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Title Page

Protocol Title:	Determination of the effect of ^{13}C-Spirulina Nitrogen content on *in-vivo* ^{13}C-Spirulina Gastric Emptying Breath Test (GEBT) Results
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Protocol Amendment Summary of Changes Table

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Amendment
Overall Rationale for the Amendment

Section # and Name	Description of Change	Brief Rationale
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1.0 STUDY SYNOPSIS

The purpose of this study is to determine whether there is a difference in the human *in vivo* response to ^{13}C-Spirulina GEBT meals manufactured using ^{13}C-Spirulina containing different levels of protein (as measured by %nitrogen).

^{13}C-Spirulina is the carbon-13 labeled substrate incorporated into the Gastric Emptying Breath Test (GEBT) test meal (100 mg of ^{13}C-Spirulina in 27 g of powdered egg). Consumption of the GEBT test meal and subsequent digestion gives rise to $^{13}CO_2$ in human breath. The rate of $^{13}CO_2$ excretion arising from the carbon-13 labeled test meal reflects a patient's gastric emptying rate.

^{13}C-Spirulina used in the GEBT is produced at Cairn Diagnostics by culturing a pure axenic inoculum of Spirulina in a growth medium enriched in carbon-13, a safe, non-radioactive stable isotope of carbon. ^{13}C-Spirulina thus produced is uniformly labeled with carbon-13 to an abundance level of approximately 99% and contains a mixture of ^{13}C-labeled protein, carbohydrates, and lipids. When metabolized, the proteins, carbohydrates and lipids in the Spirulina give rise to respiratory CO_2 that is enriched in carbon-13. The total carbon-13 content of ^{13}C-labeled Spirulina is approximately 47% by weight (dry basis). The total carbon-13 content specification for release of ^{13}C-Spirulina batches is 43% - 51% by weight carbon-13.

^{13}C-labeled Spirulina is assayed for %CHN (carbon, hydrogen, nitrogen) to further characterize each batch. The Nitrogen content is multiplied by 6.25 to approximate the protein content of the ^{13}C-Spirulina. The mean Nitrogen content of batches of ^{13}C-Spirulina released for use in GEBT test meals is 7.4% and each batch must meet a specification of 7-11% Nitrogen (44-69% protein).

In this study participants will be administered the Standard FDA-approved GEBT in which the test meals contain ^{13}C-Spirulina that has 7.9% Nitrogen content (AM). On a second occasion, a low Nitrogen GEBT in which the test meal contains ^{13}C-Spirulina with 6.4% Nitrogen content (LM) will be administered. Both sets of GEBTs have been manufactured under full cGMP's. Both tests will be administered according to the FDA-approved GEBT labeling.

The *in-vivo* results of the two independent GEBT test administrations (AM and LM) in the study cohort will be compared to determine whether there is any significant difference in *in vivo* $^{13}CO_2$ signaling. The hypothesis is that there will be no significant difference. The null hypothesis is that there will be a significant difference.

2.0 DEFINITIONS

Term/Abbreviation	Definition
Carbon-13	Carbon-13, denoted as ^{13}C, is a stable, non-radioactive, safe, naturally occurring form of carbon. Carbon-13 occurs in nature in a natural abundance of approximately 1%.
Carbon-12	Carbon-12 (^{12}C) is the most abundant, non-radioactive form of carbon in nature. Natural abundance is approximately 99%.
ABCA GIRMS	Automated Carbon Breath Analyzer Gas Isotope Ratio Mass Spectrometer. Used to analyze stable, non-radioactive isotopes of carbon, ^{13}C and ^{12}C, in QC gases and human breath samples. The ABCA GIRMS is an FDA-approved instrument for use with Cairn Diagnostics' ^{13}C-Spirulina Gastric Emptying Breath Test

Term/Abbreviation	Definition
	("GEBT"). For these purposes, the instrument measures the ratio of $^{13}CO_2/^{12}CO_2$ in QC gases and human breath.
ABCA2 GIRMS	Sercon's new model Automated Carbon Breath Analyzer-2 Gas Isotope Ratio Mass Spectrometer. Used to likewise analyze the ratio of $^{13}CO_2/^{12}CO_2$ in QC gases and human breath.
AE	Adverse Event
Delta values ($\delta^{13}C$)	Amount of ^{13}C in a sample expressed as a ratio of carbon-13 to carbon-12: $\delta^{13}C(‰) = \frac{R_B - R_S}{R_S} * 1000$ Where R_B is the ratio of $^{13}C/^{12}C$ of the sample and R_s is the ratio of $^{13}C/^{12}C$ in Pee Dee Belemnite (PDB), the reference standard for these measurements.
DOB	Delta Over Baseline i.e. the difference in delta value at any given time point and the pre-meal/baseline delta value
GEBT	Gastric Emptying Breath Test. The ^{13}C-Spirulina Gastric Emptying Breath Test ("GEBT") is an FDA-PMA approved, Class III combination drug medical device in vitro diagnostic product intended for measurements of the rate of solid phase gastric emptying and identification of gastroparesis (delayed gastric emptying).
GES	Gastric Emptying Scintigraphy
GRAS	Generally Recognized As Safe
PHI	Personal Health Information
SAE	Serious Adverse Event
WOCBP	Women of Childbearing Potential

3.0 BACKGROUND

3.1 Intended Use

The Gastric Emptying Breath Test (GEBT), to be used with the GEBT test meal, is a quantitative test intended for use in the measurement of the rate of gastric emptying of solids and to aid in the diagnosis of delayed gastric emptying (gastroparesis) in adults who are symptomatic for gastroparesis. For these purposes, the test system utilizes a Gas Isotope Ratio Mass Spectrometer (GIRMS) for the measurement of the ratio of $^{13}CO_2$ to $^{12}CO_2$ in breath samples.

The GEBT should be administered under supervision of a health care professional although no specialized facilities or specially licensed personnel are required.

3.2 Principle of the GEBT

After an overnight fast, a test meal containing non-radioactive ^{13}C-labeled Spirulina is administered to the patient. As the test meal is emptied from the stomach it is rapidly absorbed across the duodenum and metabolized giving rise to exogenous ^{13}C-labeled CO_2 which is excreted in the breath. The rate of $^{13}CO_2$ excretion in breath at any given GEBT measurement time is directly proportional to the rate of gastric emptying.

3.3 Description of the GEBT Device

Cairn's ^{13}C-Spirulina GEBT is a Class III in vitro diagnostic medical device that consists of three FDA regulated components:

- A diagnostic drug (^{13}C-Spirulina/Egg mix) that contains the active pharmaceutical ingredient (^{13}C-Spirulina) that gives rise to ^{13}C-labeled CO_2 in patients' breath when taking the test.
- A kit containing the diagnostic drug, repackaged saltine crackers, consumables used to prepare the meal, a breath collection kit (screw capped glass tubes and a straw), materials to allow return of breath samples and approved labeling (instructions for use/package insert).
- An Automated Breath Carbon Analyzer Gas Isotope Ratio Mass Spectrometer (ABCA-GIRMS) used to determine the ratio of $^{13}CO_2$ to $^{12}CO_2$ in breath samples.

3.4 Components and Pictures of ^{13}C-Spirulina GEBT Kit, Prepared Test Meal and ABCA GIRMS Instruments

The components of the ^{13}C-Spirulina GEBT are addressed below.

- ^{13}C-Spirulina Gastric Emptying Breath Test (GEBT) – Test Meal (^{13}C-Spirulina/Egg)

  Ingredients: Desugared whole eggs, Dry non-fat milk solids, Salt, Smoke Flavoring (Char Oil), ^{13}C-labeled Spirulina

  Nutritional value: Fat 8.8 g; Carb 4 g; Fiber 0 g; Protein 12g
  Energy value; 150kCal
  Net weight; 27g

- ^{13}C-Spirulina Gastric Emptying Breath Test (GEBT) – Saltine Crackers (3 packages of 2 crackers)

  Ingredients: Unbleached enriched wheat flour (wheat flour, niacin, reduced iron, thiamine mononitrate, riboflavin, folic acid), canola oil, palm oil, sea salt, malted barley flour, baking soda, yeast.
  Nutritional value; Fat 1 g; Carb 14 g; Fiber 0 g; Protein 1g
  Energy value; 80 kCal
  Net weight; 18 g

- Overall Meal Nutritional/Energy Values

  Fat 9.8g, Carb 18g, Fiber 0g, Protein, 13g, 230kCal

Table 1. Contents of ^{13}C-Spirulina GEBT Kit

Meal preparation components of Kit	Breath Sample Collection Components of Kit
1 Instructions for Use/Package Insert	1 Test Request Form
1 ^{13}C-Spirulina/Egg Meal packaged in a foil pouch with oxygen absorber	2 Blue-Capped Exetainer tubes labeled for pre-meal collection
3 packages of 2 saltine crackers re-packaged in a foil pouch with oxygen absorber	6 White-Capped Exetainer tubes labeled for post-meal collection

Meal preparation components of Kit	**Breath Sample Collection Components of Kit**
1 large (~13 fl oz/390 mL) microwaveable cooking cup	2 drinking straws
1 filling cup (small (~3.5 fl oz/~100 mL) plastic cup with pour spout for transferring water)	1 Breath tube holder
1 plastic cutlery kit (knife, fork and spoon)	1 Pre-labeled Bubble Mailer

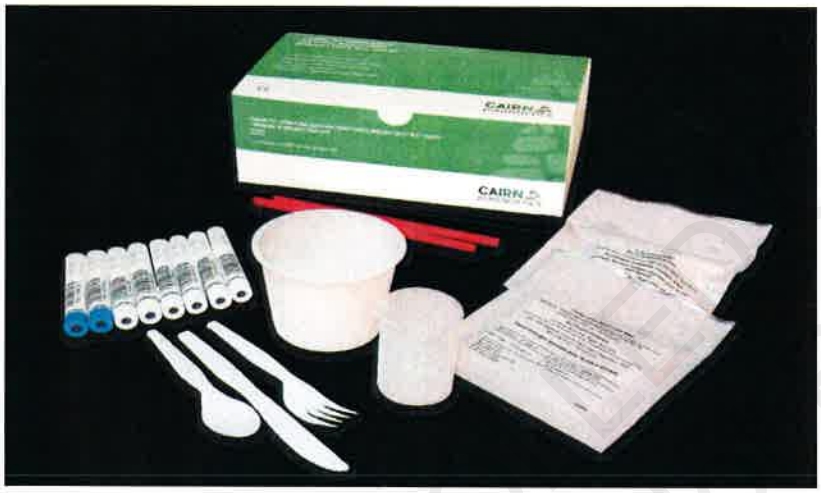

Figure 1. Contents of ^{13}C-Spirulina GEBT Kit Displayed

Figure 2. Prepared ^{13}C-Spirulina GEBT Test Meal and Breath Sample Collection Components

Figure 3. ABCA GIRMS instrument Currently Approved by FDA for Use with GEBT

3.5 Summary of Relevant Clinical Studies

The GEBT was validated in FDA-approved dual-labeled validation studies. The comparative method was a nuclear medicine procedure known as Gastric Emptying Scintigraphy ("GES"). GES is conducted by administering a radionuclide-labeled test meal to a fasting patient and measuring the rate of radiation decline with time as the stomach empties the labeled test meal. In the validation studies GEBT and GES were conducted concurrently (Mayo Clinic). GEBT demonstrated excellent agreement with diagnosis by GEBT vs. GES.

The FDA approved GEBT for commercial use in April 2015 (Pre-Market Approval PMA P110015: Gastric Emptying Breath Test). Exhibit A includes the FDA's public announcement of the approval and a copy of PMA 110015 Gastric Emptying Breath Test Letter of Approval.

GEBT has an excellent safety profile with no serious adverse events reported in pre-validation, validation and post validation studies. There have been no medical device reportable (MDR) events post approval (> 5,000 GEBT).

A recent international consensus statement recommended GEBT for use in the evaluation of gastroparesis "because of its careful validation, high concordance with scintigraphic data and FDA approval" (INTERNATIONAL CONSENSUS STATEMENT: Advances in the diagnosis and classification of gastric and intestinal motility disorders. Gastroenterology and Hepatology, Volume 15, May 2018).

GEBT is currently used in the majority of Phase II, and III pharmaceutical studies for new drugs for gastroparesis. In these studies, GEBT is used to identify gastroparetic patients for enrollment and to assess physiologic effects of new pharmacologic agents (U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research

(CDER); Gastroparesis: Clinical Evaluation of Drugs for Treatment; Guidance for Industry. July 2015, August 2019. Clinical/Medical).

Table 2 summarizes key peer-reviewed literature regarding ^{13}C-Spirulina GEBT.

Table 2. Summary of Key Peer-Reviewed Literature

Publication	Brief Description
J S Lee, M Camilleri, A R Zinsmeister, D Burton, L J Kost, P D Klein. A valid, accurate, office based non-radioactive test for gastric emptying of solids Gut 2000;46:768–773	Proof of principal study in healthy subjects showing the excellent correlation of simultaneous ^{13}C-Spirulina GEBT measurements with GES.
Viramontes B, Kim M, Camilleri M, et al. Validation of a stable isotope gastric emptying test for normal, accelerated or delayed gastric emptying. Neurogastroenterology and Motility 2001; 13:567-574	In this study GEBT and GES were conducted simultaneously in subjects with conditions of delayed, normal and accelerated emptying. Data presented in this study showed a very high correlation between GES and GEBT values ($r = 0.86$; $P < 0.0001$). GEBT had a sensitivity of 86% and specificity of 80% for detecting abnormal emptying (delayed and accelerated).
Szarka L, Camilleri M, Vella A, et al. A stable Isotope Breath Test with a Standard Meal for Abnormal Gastric Emptying of Solids in the Clinic and in Research. Clinical Gastroenterology and Hepatology 2008; 8: 635-643. (Pivotal Validation Study and basis of FDA approval)	This study validated the ^{13}C-spirulina GEBT in a prospective manner among 129 symptomatic subjects meeting the criteria for referral to GES in a tertiary clinical setting. This study also demonstrated that the normal, day-to-day intra-patient biologic variability of gastric emptying is the same as measured by GES or GEBT.

PRO-CD-005, ^{13}C-Spirulina GEBT – test meal equivalence study (Exhibit B) was designed to show the equivalence of GEBT meals manufactured by two different methods. In this study, each subject received a GEBT test meal manufactured by an original process (where lyophilized ^{13}C-Spirulina was mixed with liquid egg, lyophilized, milled and packaged) or a modified process (where lyophilized ^{13}C-Spirulina was mixed with lyophilized milled egg in a V-blender, then granulated and re-mixed prior to unit dose packaging).

4.0 STUDY GOALS AND OBJECTIVES

The purpose of this study is to establish and demonstrate by objective evidence whether there is a difference in *in vivo* ^{13}C-Spirulina GEBT results using test meals containing Spirulina with different nitrogen (protein) content.

This study will be conducted in compliance with this Protocol, GCPs, and IDE/IND regulations set forth in 21 CFR 812 and 21 CFR 312 and as applicable to this combination product.

5.0 SUBJECT SELECTION

Cairn personnel and their family and friends may volunteer to be a participant in this study. This study requires up to eighty-eight (88) participants.

5.1 Inclusion criteria

Participants are eligible to be included in the study if they meet the following criteria:

- Males and females, 18 – 85 years old at time of signing the informed consent form. Females of childbearing potential (FOCBP) must have negative urine or serum pregnancy test within 48 hours of the Gastric Emptying Breath Test.
- Ability to eat test meal and provide breath samples.

5.2 Exclusion criteria

- History or physical exam suggestive of systemic disease such as diabetes mellitus or pathophysiologic disorders such as renal failure, chronic heart disease, chronic respiratory disease, liver disease, or malabsorption syndrome.
- Symptoms consistent with delayed gastric emptying.
- History of abdominal surgery except appendectomy.
- Use of any medications that may alter gastric motility within two days of the study.
- Use of narcotics or anticholinergics within two days of the study.
- Females on hormone replacement therapy other than birth control medications.
- Receipt of an investigational drug within 4 weeks of the study.
- Pregnancy.
- Intolerance or allergy to any component of Gastric Emptying Breath Test meal
- History of neurologic or psychiatric disorders.

5.3 Participant Meal and Dietary Restrictions

Participants will fast for at least 8 hours (preferably overnight) prior to administration of the GEBT and during the GEBT (with the exception of eating the test meal). Alcohol should not be consumed within 8 hours prior to testing. Participants may consume a small amount of water up to 1 hour before the test, but not more than 4 fl oz.

Subjects should not smoke/use tobacco products (e.g. chewing tobacco, nicotine gum) before or during administration of the GEBT.

5.4 Participant Activity Restrictions

Participants will abstain from strenuous activity for at least 8 hours prior to administration of the GEBT. Participants may participate in light activities during administration of the GEBT (e.g. watching television, reading, using restroom) but otherwise will remain comfortably seated in the test administration location.

6.0 STUDY PROCEDURES/RESEARCH METHOD

6.1 Study Scheme

Visit 1: For each potential participant, perform a screening visit via a telehealth conference or at the GEBT testing facility (Cairn Diagnostics facility in Brentwood, Tennessee) where the study is explained to the participant. Ensure that the participant is eligible to participate in the study. Answer any questions about the GEBT test procedure and ask the participant to provide

consent to participate in the study (See SPEC-CD-031, Informed Consent). Participants are free to leave the test site upon completion of the screening visit.

Visit 2: Within two weeks of visit 1, participants will return to the test site at which time the GEBT will be administered. Participants averse to returning to the test site because of COVID concerns may be administered the GEBT via Cairn Diagnostics' visually supervised GEBT TeleAdministration procedure provided the participant has been qualified to do so at Visit 1. FOCBP will produce evidence of a negative pregnancy test taken within the previous 48 hours of the scheduled GEBT. After confirmation that the participant has met the fasting and dietary restriction requirements, the participant will take the GEBT using either the FDA- approved ^{13}C-Spirulina GEBT meal (AM) or the alternative, low nitrogen ^{13}C-Spirulina GEBT meal (LM) according to instructions in the approved labeling for ^{13}C-Spirulina GEBT. Breath samples will be collected prior to consumption of the test meal and after completion of the meal at 45, 90, 120, 150, 180, and 240-minute timepoints.

Follow up contact (e.g. phone call, email, in-person, etc) will be made with the participant within one week following the GEBT administration to assess whether there are any post-administration adverse events.

Visit 3: The procedure for Visit 3 is the same as for Visit 2 except that a different test meal will be administered than was administered at Visit 2. The time period between Visit 2 and Visit 3 will be no less than 48 hours and no greater than 3 weeks (note: the washout period for GEBT is 24 hours).

Visit 4: Follow up contact (e.g. phone call, email, in-person, etc) will be made with the participant within one week following the GEBT administration to assess whether there are any post-administration adverse events and to complete subject's participation in the study.

Table 3. Schedule of Activities (SoA)

Procedure	Screening (Visit 1)	Visit 2	Visit 3	Follow-up (Visit 4)	Notes
Informed Consent	X				
Inclusion and Exclusion Criteria	X				
Serum or urine pregnancy test		X	X		
Administration of ^{13}C-Spirulina GEBT		X	X		Each subject will take GEBT twice – once using the approved ^{13}C-Spirulina/Egg meal and once using the low nitrogen ^{13}C-Spirulina/Egg meal
AE review				X	
SAE review				X	

6.2 Description of ^{13}C-Spirulina used in the GEBT

Spirulina is a blue-green microalga with a history of use in the human diet as a source of protein. Spirulina (*Arthospira platensis)* has been recognized as a GRAS substance since 2003 (FDA, GRN 000127) and is currently consumed in the US as a dietary supplement at doses of 3 to 4.5 grams per day. The safety of Spirulina has been established through centuries of food use and numerous toxicology studies. Acute, subchronic, chronic, teratogenicity and

mutagenicity studies have shown no toxic effect. Carbon-13 stable isotope labeling is inherently safe as 1.1% of all carbon in our bodies and in the food that we eat is carbon-13.

The ^{13}C-Spirulina used in the GEBT is produced at Cairn Diagnostics by culturing a pure axenic inoculum of *Arthrospira platensis* (commonly known as Spirulina) in a growth medium enriched in carbon-13, a non-radioactive stable isotope of carbon. ^{13}C-Spirulina thus produced is uniformly labeled with carbon-13 to an abundance level of approximately 99% and contains a mixture of protein, starch and lipids. When metabolized, the proteins, carbohydrates and lipids of the Spirulina give rise to respiratory CO_2 that is enriched in carbon-13.

Incorporation of ^{13}C-labeled Spirulina into an egg meal provides a means to assess the emptying of the solid phase of the GEBT test meal. Thus, carbon-13 can only be released from the algal cells as the egg meal is emptied, the cells are digested, and the ^{13}C-labeled substrates (from proteins, carbohydrates, and lipids) absorbed.

^{13}C-Spirulina is cultured according to a validated process that requires specific temperature, light, oxygen, pH and rotation rates at the different stages of growth. After harvest, ^{13}C-Spirulina undergoes release testing, to ensure consistency of the material from batch to batch: %CHN (carbon, hydrogen, nitrogen) and %^{13}C specifications confirm the equivalence of the ^{13}C-Spirulina. %CHN specifications for ^{13}C-Spirulina were originally set based on results of < 10 batches of ^{13}C-Spirulina. Cairn has since grown almost 30 more batches of ^{13}C-Spirulina according to the validated method and has determined that the average %nitrogen in all batches is lower than would have been predicted by the initial 10 batches.

Cairn would like to use the results of this study and the data from the additional 30 batches of ^{13}C-Spirulina to more appropriately set the specification for CHN for ^{13}C-Spirulina produced according to the validated method. However, in order to do this, Cairn must first prove that there is no difference in the *in vivo* response to ^{13}C-Spirulina with a nitrogen content less than the lower limit of the currently approved specification.

6.3 Administration of ^{13}C-Spirulina GEBT

^{13}C-Spirulina GEBT meals will be administered to participants according to the instructions enclosed in the GEBT kit in the approved package insert. Participants may be provided with results from their GEBT using the approved (validated) meal, if they so desire. Results of the GEBT using the low Nitrogen ^{13}C-Spirulina meal will not be provided to participants, because it is as yet unknown whether they will be accurate.

6.4 Analysis of Breath samples

Breath samples will be analyzed using Cairn's approved ABCA-GIRMS instrument according to SOP-CD-005, Breath Test Processing.

6.5 Experimental Design and Analyses

6.5.1 Study Design

Cairn will conduct a single-center, open-label, two-sided test of equivalence of normally distributed continuous variables of unknown variance in a group-sequential, two-treatment, two-period cross-over trial. In this design, X_i denotes the difference between measured gastric emptying results produced from the low %N ^{13}C-Spirulina meal (LM) and the FDA Approved %N ^{13}C-Spirulina meal (AM) [$X_i = (LM)_i - (AM)_i$] for the i^{th} subject receiving the low %N ^{13}C-Spirulina meal first. Similarly, Y_i is defined as $Y_i = (LM)_i - (AM)_i$ for the i^{th} subject receiving the FDA Approved %N ^{13}C-Spirulina meal first. After each stage (k = 1 to 2) of the two-stage group-sequential design, the test statistic (θ) is evaluated as the average difference between results

from the low %N ^{13}C-Spirulina meal and the approved %N ^{13}C-Spirulina meal results for the patients tested:

$$\hat{\theta}^{(k)} = \frac{\left(\overline{X}^{(k)} + \overline{Y}^{(k)}\right)}{2}$$

Following the statistical procedure recommended by Jennison and Turnbull for this two-sided test of equivalence, we construct a two-sided equivalence test satisfying:

$$Pr_{\Theta=\pm\delta}\{\text{declare equivalence}\} \leq \beta$$

for specified δ (the Margin of Equivalence defined as the maximum difference in test meal response considered to be inconsequential, see Section 6.5.4), and β. The probability, β, represents the "consumer's risk" since wrongly declaring equivalence could lead to inaccurate GEBT results using the low %N ^{13}C-Spirulina test meal.

We also wish to satisfy the error condition:

$$Pr_{\Theta=0}\{\text{do not declare equivalence}\} \leq \alpha$$

The probability, α, is the "manufacturer's risk" in that declaring a meal difference when in fact there is no difference, would result in an unnecessary and expensive revalidation.

Detailed statistical calculations based on these operational requirements are summarized in Section 6.5.4.

6.5.2 Margin of Equivalence

Cairn proposes using the same "margin of equivalence" (δ) for the GEBT as was used in PRO-CD-005, the crossover trial originally used to demonstrate equivalence of original and modified process ^{13}C-Spirulina GEBT meals. Refer to PRO-CD-005 (Exhibit B) for a full description of the modeling that resulted in the margin of equivalence for that study.

The GEBT "margin of equivalence", δ = 3.0 min^{-1} (kPCD), which is the absolute value of the smallest bias at which the point estimate of average concordance, at both the 90 and 120 minute time points, is less than the lower limit of the 95% confidence interval of the 0-bias concordance (96.8).

For simplicity, and because based on the shape of GEBT curves they are likely to show the largest differences in kPCD values (especially in subjects with normal gastric emptying), the 90 and 120 minute timepoints will be used to determine the difference in the meal response.

6.5.3 Sample Size

Cairn proposes using the same sample sizes to demonstrate equivalence (or otherwise) in this study as were used in PRO-CD-005, Equivalence study. Refer to PRO-CD-005 (Exhibit B) for a full description of the justification of sample sizes.

Table 4. Margins of Equivalence

Parameter	GEBT
δ (margin of equivalence)	3.0
σ_D (StdDev of differences)	9.62

Table 5. Enrollment Plan for the LM/AM crossover trial

Stage	Nx_k *	Ny_k **	Total Subjects	Total GEBT Results
1	22	22	44	88
2 (if necessary)	22	22	44	88
Totals	44	44	88	176

* Nx_k is the number of subjects who will receive the low %N ^{13}C-Spirulina meal (LM) first.

** Ny_k is the number of subjects who will receive the Approved %N ^{13}C-Spirulina meal (AM) first.

6.5.4 Statistical Calculations

Equations in this section can be simplified by noting that the trial has been planned such that the number of subjects receiving the low %N ^{13}C-Spirulina meal (LM) first equals the number of subjects receiving the low %N ^{13}C-Spirulina meal (LM) second at each stage of the trial. Thus, $n_{Xk} = n_{Yk} = n_k$.

We defined the average difference in results at each stage of the trial to be:

$$\hat{\theta}^{(k)} = \frac{\left(\overline{X}^{(k)} + \overline{Y}^{(k)}\right)}{2} \qquad \text{with } Var(\hat{\theta}^{(k)}) = \frac{\sigma_D^2}{2n_k}$$

Note that $\overline{X}^{(k)}$ and $\overline{Y}^{(k)}$ represent the means of data accumulated through stage k.

The observed, one-sided t-statistics calculated after each stage of the trial are:

$$\left(T_k^+\right)_{obs} = \frac{\left(\hat{\theta}^{(k)} - \delta\right) * \sqrt{2n_k}}{s_k} \qquad \text{and} \qquad \left(T_k^-\right)_{obs} = \frac{\left(\hat{\theta}^{(k)} + \delta\right) * \sqrt{2n_k}}{s_k}$$

$$\text{with } s_k = \sqrt{\frac{\left(\sum_{i=1}^{n_{Xk}} (X_i - \overline{X}_k)^2 + \sum_{i=1}^{n_{Yk}} (Y_i - \overline{Y}_k)^2\right)}{(n_{Xk} + n_{Yk} - 2)}}$$

Now, with h_k and g_k defined for each k^{th} stage of K stages in terms of the prescribed Δ value and numerically calculated constants $\widetilde{C}_{W1}(K, \alpha, \beta, \Delta)$ and $\widetilde{C}_{W2}(K, \alpha, \beta, \Delta)$ which ensure Type I and Type II error conditions for the group sequential tests:

[Note: Constants $\widetilde{C}_{W1}$ and $\widetilde{C}_{W2}$ are listed in Jennison & Turnbull, 2000, Table 5.1]

$$h_k = -\left(\widetilde{C}_{W1} + \widetilde{C}_{W2}\right) * (k/K)^{1/2} + \widetilde{C}_{W1}(k/K)^{(\Delta - 1/2)} \qquad \text{and} \qquad g_k = -\widetilde{C}_{W2}(k/K)^{(\Delta - 1/2)}$$

we perform the two one-sided t-tests after each stage of the trial and proceed according to the following rules:

For a two-stage, group sequential trial, after stage 1,

If $\left(T_k^+\right)_{obs} \geq t_{\nu_k,1-\Phi(h_k)}$ **OR** $\left(T_k^-\right)_{obs} \leq -t_{\nu_k,1-\Phi(h_k)}$ **STOP, Reject Equivalence**

If $\left(T_k^+\right)_{obs} < t_{\nu_k,1-\Phi(g_k)}$ **AND** $\left(T_k^-\right)_{obs} > -t_{\nu_k,1-\Phi(g_k)}$ **STOP, Declare Equivalence**

Otherwise, continue to stage 2.

After stage 2,

If $\left(T_K^+\right)_{obs} \geq t_{\nu_K,1-\Phi(h_K)}$ **OR** $\left(T_K^-\right)_{obs} \leq -t_{\nu_K,1-\Phi(h_K)}$ **STOP, Reject Equivalence**

Otherwise, **STOP, Declare Equivalence**

In these equations, the $t_{\upsilon_k,(1-\Phi(z))}$ critical values refer to the Student t-deviate corresponding to ν_k degrees of freedom and probability (1-Φ(z)) where Φ(z) is to the standard normal cumulative distribution function.

6.6 End of Study

- Participants may withdraw from the study at any time, without prejudice.
- An authorized investigator may discontinue any test subject at any time if medically indicated or in the best interest of the individual.
- Participants who do not complete all components of the testing and compliance procedures will be excluded from the statistical analysis.
- Withdrawn participants may be replaced to achieve the specified number of subjects.
- The trial may be terminated prematurely if, in the judgment of Cairn's Medical Director, the severity or frequency of adverse events so warrants.

Each participant is considered to have completed the study when they have completed both GEBT test administrations and the AE review follow-up and all collected breath samples have been analyzed on the ABCA-GIRMS systems.

The end of the study is defined as the date on which all of the participants' breath samples have been analyzed on the ABCA-GIRMS and all follow-up (AE review) has been completed.

7.0 RISK/SAFETY INFORMATION

7.1 Established Contraindications, Warnings and Precautions of GEBT

The following contraindications, warnings and precautions have been established for the ^{13}C-Spirulina GEBT in FDA-approved labeling:

- Individuals with known hypersensitivity to Spirulina, egg, milk or wheat allergens should avoid the GEBT
- Because the GEBT is an indirect multi-compartmental method of measuring gastric emptying, GEBT results may be inaccurate in individuals compromised with significant small bowel, pancreatic, liver, and/or lung disease. Consequently, GEBT should not be administered to patients with pulmonary dysfunction (e.g. COPD) and/or small bowl malabsorption.
- Individuals with severe lactose intolerance may wish to avoid the GEBT, as the test meal contains a small amount of lactose, approximately 2.7 grams.

- The performance characteristics for individuals under the age of eighteen (18) years have not been established for this test.
- The performance characteristics for pregnant women have not been established for this test.
- False positive and false negative results can occur with this test.
- Follow the directions for collecting breath samples carefully. Errors in the timing and/or procedures for collecting breath samples may affect test results and necessitate retesting.
- The GEBT should not be performed in individuals who have taken medications known to influence the rate of gastric emptying (e.g. erythromycin, metoclopramide, opiates and anticholinergics) within three (3) days prior to testing. Individuals should stop such medications only after consulting with and obtaining approval from their attending physician or the physician ordering the test.
- Fasting serum glucose levels of diabetic subjects should be checked before administration of GEBT and the test should only be administered to subjects with a fasting serum glucose level of <275mg/dl.
- After 24 hours there is no residual $^{13}CO_2$ signal in the breath arising from the ^{13}C label contained in the GEBT meal; thus, the GEBT may be administered as frequently as every 24 hours.
- The GEBT should not be administered within 24 hours (or the relevant washout period) of other ^{13}C breath tests (e.g. the ^{13}C-Urea breath test for H. pylori).

7.2 Potential Risk to Participant Associated with this Study

This study involves the following risks:

- Allergic reactions such as rash, itching, hives or problems breathing are a possibility if the participant were unknowingly and severely allergic to the GEBT test meal ingredients. The ingredients are 100 mg Spirulina, 27 grams of dried scrambled eggs (with nonfat milk added), saltine crackers (containing wheat) and water.

Any unanticipated problems will be reported to the IRB within ten (10) calendar days of being reported.

8.0 MONITORING AND REPORTING OF ADVERSE EVENTS/SERIOUS ADVERSE EVENTS

An Adverse Event (AE) is any untoward medical occurrence in the participant associated with administration of GEBT, whether or not considered related to GEBT.

A Serious Adverse Event (SAE) is defined as any untoward medical occurrence that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent disability/incapacity

AE's will be reported by the participant (or, when appropriate, by a caregiver, a surrogate of the participant or the participant's legally authorized representative).

The investigator and any qualified designees are responsible for detecting, documenting and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the GEBT test procedure or study procedures that caused the participant to discontinue the study.

8.1 Time Period and Frequency for Collecting AE and SAE Information

All AEs or SAEs will be collected from the day of each GEBT test administration until the follow-up visit as outlined in Table 3, Schedule of Activities.

In the event any serious adverse events are reported, or observed during the GEBT administration procedure, whether or not attributable to the GEBT test procedure, the event will be reported within 24 hours to the IRB and to Cairn's medical director.

The following information will be provided in writing: study protocol number, patient's identification code, date of birth, date and nature of the serious adverse event and the causality assessment. The report of an SAE will be completed and signed by the next working day.

8.2 Intensity of an Event

The intensity/severity of an event will be classified as follows:

- Mild: that is an awareness of sign or symptom, but easily tolerated
- Moderate: that is discomfort of sign or symptom, but easily tolerated
- Severe: at least partially incapacitating (or restricting usual activity)

8.3 Relationship to Study Procedures

Adverse events will be considered associated with the study procedure if the attribution is possible, probable or very likely. The relationship can be classified as follows:

- Not related: an adverse event that is not related to the study procedure
- Doubtful: an adverse event for which an alternative explanation is more likely
- Possible: an adverse event, which might be due to the study procedure
- Very likely: an adverse event which is listed as a possible adverse reaction and cannot be reasonable explained by an alternative explanation
- Unknown: it is not possible to assign the reaction to any of the above categories because of insufficient, pending or contradictory information

8.4 Follow-up of AEs and SAEs

After the initial AE/SAE report, Cairn's medical director will follow the participant at subsequent visits, contacts, etc. All SAEs and non-serious AEs of special interest will be followed until resolution, stabilization, the event is otherwise explained, or the participant is lost to follow-up.

If an adverse event results in withdrawal, the patient will be followed up until the cause of the event is established, if possible, and the outcome resolved, or the patient's condition stabilized.

8.5 Regulatory Reporting Requirements for SAEs

Prompt notification of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study intervention under clinical investigation are met.

Cairn has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of GEBT. Cairn will comply with country-specific (U.S. FDA) and IRB-specific regulatory requirements relating to safety reporting.

9.0 STUDY OVERSIGHT

Samantha Bouldin, PhD, or another qualified Cairn employee specifically designated by Cairn's medical director, Alex Ryder, MD, PhD, will provide oversight of this study protocol and supervise all study activities. The designee will ensure that all personnel associated with the study are adequately trained in the study protocol and delegations of study duties will be given to the appropriately trained personnel.

Cairn Diagnostics Quality Assurance and Compliance personnel will assure:

- The study protocol is being followed as approved by Cairn Diagnostics and the IRB
- Informed consent is being obtained before GEBT test administration is conducted
- Accurate, complete and current records are being maintained
- Responsibilities have not been delegated to unspecified or untrained personnel

10.0 PRODUCT STORAGE AND ACCOUNTABILITY

GEBT kits will be distributed to Cairn personnel as needed for the course of the study. The kits are stored in a controlled, limited access area at controlled room temperature (20°C-25°C). The kits have an expiry date and should not be used beyond the expiration date displayed on the GEBT test kit box. Collected breath samples should be stored at room temperature. Kit receipt, usage or destruction will be recorded in the GEBT kit inventory to assure all kits are accounted for during the study.

11.0 MEASURES TO MINIMIZE BIAS: RANDOMIZATION

This is an open-labeled study; potential bias will be reduced by the following steps:

Subject Number	FDA-approved GEBT(AM)	Low nitrogen GEBT(LM)
001	Visit 2	Visit 3
002	Visit 3	Visit 2
003	Visit 3	Visit 2
Etc	Etc	Etc

12.0 DATA MANAGEMENT

12.1 Breath Sample Analysis

Perform sample analysis on the participant(s) breath samples using the ABCA GIRMS system according to SOP-QC-017, Operation, Calibration and Maintenance – ABCA GIRMS. Analysis will be conducted by trained and qualified clinical laboratory personnel with verified and documented training appropriate for operation of the ABCA GIRMS systems.

12.2 ^{13}C-Spirulina GEBT reporting

Prepare reports for each ^{13}C-Spirulina GEBT according to Cairn SOP-CD-005, Breath Test Processing. Enter data from each individual report into a spreadsheet for statistical analysis.

12.3 Sample retention

Retain all participant breath samples collected for at least 28 days after the sample collection date recorded on the GEBT Test Request Form. Dispose of the samples according to SOP-CD-021, GEBT Breath Sample Accessioning and Chain of Custody.

12.4 Data Retention

- Maintain data as hard copies and scan and store electronic copies on Cairn's server.
- Review all data generated during execution of the validation protocol for completeness and accuracy in accordance with SOP-QC-030, Data Generation and Review.

12.5 Statistical Analysis

See section 6.5 of this document.

12.6 Reporting

Collate the results of the executed protocol, including all associated data, and document the completion of the protocol in a report. The report must be approved by the same individuals who approved the associated protocol.

13.0 IRB REVIEW/ETHICS/INFORMED CONSENT

13.1 Regulatory and Ethical Considerations

This study will be conducted in accordance with this protocol and in accordance with:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organization of Medical Sciences International Ethical Guidelines
- Applicable ICH Good Clinical Practice Guidelines
- Applicable U.S. laws and regulations

This protocol, protocol amendments, Informed Consent Form and other relevant documents (e.g. advertisements) must be submitted to an IRB/IEC and reviewed and approved by the IRB/IEC before initiation of this study.

Any amendments to this protocol will be submitted for IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

13.2 Informed Consent Process

Obtain a signed consent from the participant prior to any participation in study activities. Participants may ask questions and/or withdraw from the study at any time.

A copy of the Informed Consent Form (SPEC-CD-031) must be provided to the participant.

14.0 CONFIDENTIALITY

Assign each participant a unique identifier. Any participant's data sets that are transferred to any other party will contain the identifier only; participant names or any information which would make the participant identifiable will not be transferred.

The following steps ensure that information about the participant is kept confidential, and will protect it from unauthorized disclosure, tampering or damage:

1) Verbal PHI Safeguards
    - Verbal discussions regarding the participant's information shall be performed in locations that are as private as possible and reasonable measures, such as lowering voices or asking unauthorized personnel to step away, shall be taken to assure unauthorized personnel to not overhear conversation
2) Written PHI Safeguards
    - All documents shall be stored in Cairn's GEBT accessioning room in locked file cabinets with limited accessibility and only authorized staff have access.
    - Clinical laboratory management shall identify and document issuance of keys to personnel that are allowed access to Participant records.

15.0 INTENDED USE OF DATA

Data obtained in this study will be used to determine whether there is justification to change the nitrogen specification for ^{13}C-Spirulina used in the ^{13}C-Spirulina GEBT and may be submitted to FDA as a supplement to PMA P110015.

16.0 REFERENCES

A. J S Lee, M Camilleri, A R Zinsmeister, D Burton, L J Kost, P D Klein. A valid, accurate, office based non-radioactive test for gastric emptying of solids Gut 2000;46:768–773
B. Viramontes B, Kim M, Camilleri M, et al. Validation of a stable isotope gastric emptying test for normal, accelerated or delayed gastric emptying. Neurogastroenterology and Motility 2001; 13:567-574
C. Szarka L, Camilleri M, Vella A, et al. A stable Isotope Breath Test with a Standard Meal for Abnormal Gastric Emptying of Solids in the Clinic and in Research. Clinical Gastroenterology and Hepatology 2008; 8: 635-643.
D. Jennison C and Turnbull BW. Group Sequential Trials with Applications to Clinical Trials. Chapman and Hall/ CRC. 2000.

17.0 EXHIBITS

A. PMA P110015 Approval Letter and FDA Press Release
B. PRO-CD-005, Clinical Study Report: ^{13}C-Spirulina GEBT – Test Meal Equivalence Study
C. COVID19 Transmission Mitigation Plan
D. Study Participant Health Questionnaire
E. Contraceptive Guidance & Collection of Pregnancy Information

18.0 REVISION HISTORY

Revision Level	Brief Description of Revision(s)	Effective Date
00	Initial document	See first page of this document.

19.0 PRINCIPAL INVESTIGATOR AGREEMENT:

By signing this protocol, I commit to conducting the clinical investigation in accordance with the procedure all requirements of the investigational plan, IDE regulations, other applicable regulations of the FDA, and any conditions of approval imposed by the Institutional Review Board (IRB) or FDA. I agree to abide by all of the responsibilities of investigators addressed in 21 CFR Part 812, Subpart E and Subpart G.

	NAME (Print)	SIGNATURE	DATE
Principal Investigator:	Alex Ryder	Alex Ryder	7-15-20

20.0 EXHIBIT A: PMA P110015 APPROVAL LETTER AND FDA PRESS RELEASE

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

April 6, 2015

MR. KERRY BUSH
PRESIDENT
ADVANCED BREATH DIAGNOSTICS, LLC
105 WESTPARK DRIVE, SUITE 150
BRENTWOOD, TN 37027

Re: P110015
Gastric Emptying Breath Test (GEBT)
Filed: July 11, 2012
Amended: September 18, 2012, December 26, 2012, April 5, 2013, September 19, 2013, May 15, 2014
Procode: PGE

Dear Mr. Bush:

The Center for Devices and Radiological Health (CDRH) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the Gastric Emptying Breath Test (GEBT). This device, to be used with the GEBT test meal, is intended for use in the measurement of the rate of gastric emptying of solids and as an aid in the diagnosis of delayed gastric emptying (gastroparesis) in adult humans who are symptomatic for gastoparesis. For these purposes, the test system utilizes a Gas Isotope Ratio Mass Spectrometer (GIRMS) for the measurement of the ratio of $^{13}CO_2$ to $^{12}CO_2$ in breath samples. The GEBT procedure should be administered under supervision of a health care professional although no specialized facilities or specially licensed personnel are required. We are pleased to inform you that the PMA is approved. You may begin commercial distribution of the device in accordance with the conditions of approval described below.

The sale and distribution of this device are restricted to prescription use in accordance with 21 CFR 801.109 and under section 515(d)(1)(B)(ii) of the Federal Food, Drug, and Cosmetic Act (the act). FDA has determined that this restriction on sale and distribution is necessary to provide reasonable assurance of the safety and effectiveness of the device. Your device is therefore a restricted device subject to the requirements in sections 502(q) and (r) of the act, in addition to the many other FDA requirements governing the manufacture, distribution, and marketing of devices.

Expiration dating for this device has been established and approved at three years for 20 °C - 25 °C (68 °F – 77 °F) with excursions permitted to 15 °C - 30 °C (59 °F - 86 °F). This is to advise you that the protocol you used to establish this expiration dating is considered an approved protocol for the purpose of extending the expiration dating as provided by 21 CFR 814.39(a)(7).

Continued approval of this PMA is contingent upon the submission of periodic reports, required

under 21 CFR 814.84, at intervals of one year (unless otherwise specified) from the date of approval of the original PMA. Two copies of this report, identified as "Annual Report" and bearing the applicable PMA reference number, should be submitted to the address below. The Annual Report should indicate the beginning and ending date of the period covered by the report and should include the information required by 21 CFR 814.84. This is a reminder that as of September 24, 2014, class III devices are subject to certain provisions of the final UDI rule. These provisions include the requirement to provide a UDI on the device label and packages (21 CFR 801.20), format dates on the device label in accordance with 21 CFR 801.18, and submit data to the Global Unique Device Identification Database (GUDID) (21 CFR 830 Subpart E). Additionally, 21 CFR 814.84 (b)(4) requires PMA annual reports submitted after September 24, 2014, to identify each device identifier currently in use for the subject device, and the device identifiers for devices that have been discontinued since the previous periodic report. It is not necessary to identify any device identifier discontinued prior to December 23, 2013. For more information on these requirements, please see the UDI website, http://www.fda.gov/udi.

In addition to the above, and in order to provide continued reasonable assurance of the safety and effectiveness of the device, the Annual Report must include, separately for each model number (if applicable), the number of devices sold and distributed during the reporting period, including those distributed to distributors. The distribution data will serve as a denominator and provide necessary context for FDA to ascertain the frequency and prevalence of adverse events, as FDA evaluates the continued safety and effectiveness of the device.

Be advised that the failure to conduct any such study in compliance with the good clinical laboratory practices in 21 CFR part 58 (if a non-clinical study subject to part 58) or the institutional review board regulations in 21 CFR part 56 and the informed consent regulations in 21 CFR part 50 (if a clinical study involving human subjects) may be grounds for FDA withdrawal of approval of the PMA. In addition, the results from any post approval study should be included in the labeling as these data become available. Any updated labeling must be submitted to FDA in the form of a PMA Supplement. For more information on post-approval studies, see the FDA guidance document entitled, "Procedures for Handling Post-Approval Studies Imposed by PMA Order" (http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm070974.htm).

Before making any change affecting the safety or effectiveness of the device, you must submit a PMA supplement or an alternate submission (30-day notice) in accordance with 21 CFR 814.39. All PMA supplements and alternate submissions (30-day notice) must comply with the applicable requirements in 21 CFR 814.39. For more information, please refer to the FDA guidance document entitled, "Modifications to Devices Subject to Premarket Approval (PMA) - The PMA Supplement Decision-Making Process" (www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm089274.htm).

You are reminded that many FDA requirements govern the manufacture, distribution, and marketing of devices. For example, in accordance with the Medical Device Reporting (MDR)

regulation, 21 CFR 803.50 and 21 CFR 803.52, you are required to report adverse events for this device. Manufacturers of medical devices, including in vitro diagnostic devices, are required to report to FDA no later than 30 calendar days after the day they receive or otherwise becomes aware of information, from any source, that reasonably suggests that one of their marketed devices:

1. May have caused or contributed to a death or serious injury; or

2. Has malfunctioned and such device or similar device marketed by the manufacturer would be likely to cause or contribute to a death or serious injury if the malfunction were to recur.

Additional information on MDR, including how, when, and where to report, is available at www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm.

In accordance with the recall requirements specified in 21 CFR 806.10, you are required to submit a written report to FDA of any correction or removal of this device initiated by you to: (1) reduce a risk to health posed by the device; or (2) remedy a violation of the act caused by the device which may present a risk to health, with certain exceptions specified in 21 CFR 806.10(a)(2). Additional information on recalls is available at www.fda.gov/Safety/Recalls/IndustryGuidance/default.htm.

CDRH does not evaluate information related to contract liability warranties. We remind you; however, that device labeling must be truthful and not misleading. CDRH will notify the public of its decision to approve your PMA by making available, among other information, a summary of the safety and effectiveness data upon which the approval is based. The information can be found on the FDA CDRH Internet HomePage located at www.fda.gov/MedicalDevices/ProductsandMedicalProcedures/DeviceApprovalsandClearances/PMAApprovals/default.htm. Written requests for this information can also be made to the Food and Drug Administration, Dockets Management Branch, (HFA-305), 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. The written request should include the PMA number or docket number. Within 30 days from the date that this information is placed on the Internet, any interested person may seek review of this decision by submitting a petition for review under section 515(g) of the act and requesting either a hearing or review by an independent advisory committee. FDA may, for good cause, extend this 30-day filing period.

Failure to comply with any post-approval requirement constitutes a ground for withdrawal of approval of a PMA. The introduction or delivery for introduction into interstate commerce of a device that is not in compliance with its conditions of approval is a violation of law.

You are reminded that, as soon as possible and before commercial distribution of your device, you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form. Final printed labeling that is identical to the labeling approved in draft form will not routinely be reviewed by FDA staff when accompanied by a cover letter stating that the final printed labeling is identical to the labeling approved in draft form. If the final printed labeling is not identical, any changes from the final draft labeling should be highlighted and explained in the

amendment.

All required documents should be submitted in 6 copies, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

U.S. Food and Drug Administration
Center for Devices and Radiological Health
PMA Document Control Center – WO66-G609
10903 New Hampshire Avenue
Silver Spring, MD 20993-0002

If you have any questions concerning this approval order, please contact Sunita Shukla at 301-796-6406.

Sincerely yours,

Alberto Gutierrez -S

Alberto Gutierrez
Director
Office of In Vitro Diagnostics and
Radiological Health
Center for Devices and
Radiological Health

FDA News Release

FDA approves breath test to aid in diagnosis of delayed gastric emptying

Test can be performed in a general clinical setting; does not require radioactive material

For Immediate Release

April 6, 2015

Release

Español (/NewsEvents/Newsroom/ComunicadosdePrensa/ucm441610.htm)

The U.S. Food and Drug Administration today approved the Gastric Emptying Breath Test (GEBT), a new non-invasive test to aid in the diagnosis of delayed gastric emptying, known as gastroparesis.

Current tests used to diagnose gastroparesis typically involve the use of a small amount of radioactive material or imaging equipment, so testing must be conducted in specialized outpatient centers. The GEBT can be used in broader settings.

"The GEBT is another option for aiding in the diagnosis of gastroparesis," said Alberto Gutierrez, Ph.D., director of the Office of In Vitro Diagnostics and Radiological Health in the FDA's Center for Devices and Radiological Health. "It can be performed in any clinical setting since it does not require the health care professionals administering the test to undergo special training or to take special precautions related to radiation emitting compounds."

Gastroparesis is a disorder that slows or stops the movement of food from the stomach to the small intestine when muscles in the stomach are not contracting properly. It is caused by damage to the vagus nerve that controls the muscles of the stomach and small intestine, often as a result of intestinal surgery, neurological diseases such as Parkinson's disease and multiple sclerosis, or high blood glucose levels due to diabetes. If left untreated, gastroparesis can lead to problems such as severe dehydration due to persistent vomiting, difficulty managing blood sugar levels in people with diabetes, and malnutrition due to poor absorption of nutrients or a low caloric intake.

The GEBT, conducted over a four-hour period after an overnight fast, is designed to show how fast the stomach empties solids by measuring carbon dioxide in a patient's breath. Patients have baseline breath tests conducted at the beginning of the test. They then eat a special test meal that includes a scrambled egg-mix and Spirulina platensis, a type of protein that has been enriched with carbon-13, which can be measured in breath samples.

Carbon-13 is a naturally existing non-radioactive form of the common element carbon-12. Both carbon-12 and a very small amount of carbon-13 are normally found in exhaled carbon dioxide. By adding carbon-13 to the test meal, the GEBT can determine how fast the stomach empties the meal by measuring the ratio of carbon-13 to carbon-12 collected in breath samples at multiple time points after the meal is consumed compared to baseline.

To support the safety and effectiveness of the GEBT, researchers conducted a clinical study using data from 115 participants who would typically undergo a gastric emptying test. All participants underwent testing with both the GEBT and gastric scintigraphy, the standard of care for measuring gastric emptying that requires ingestion of a test meal containing a radioactive material. Researchers compared diagnostic results from both the GEBT and scintigraphy and found that GEBT results agreed with scintigraphy results 73-97 percent of the time when measured at various time points during the test.

No deaths or serious adverse events occurred during clinical studies. Some study participants reported nausea and stomach discomfort during the test. People with hypersensitivity to Spirulina, egg, milk or wheat allergens should avoid the GEBT. The test also should not be administered to people with certain lung diseases or conditions that cause small bowel malabsorption.

The GEBT is manufactured by Advanced Breath Diagnostics, based in Brentwood, Tenn.

The FDA, an agency within the U.S. Department of Health and Human Services, protects the public health by assuring the safety, effectiveness, and security of human and veterinary drugs, vaccines and other biological products for human use, and medical devices. The agency also is responsible for the safety and security of our nation's food supply, cosmetics, dietary supplements, products that give off electronic radiation, and for regulating tobacco products.

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Inquiries

Media

Eric Pahon (mailto:eric.pahon@fda.hhs.gov)
240-402-4177

Consumers

888-INFO-FDA

Related Information

- NIH: National Digestive Diseases Information Clearing House: Gastroparesis (http://digestive.niddk.nih.gov/ddiseases/pubs/gastroparesis/)

Follow FDA

21.0 EXHIBIT B. PRO-CD-005-03, CLINICAL STUDY REPORT: ^{13}C-SPIRULINA GEBT – TEST MEAL EQUIVALENCE STUDY

Version 21FEB2011 ABD, LLC – CONFIDENTIAL

SECTION 1 TITLE PAGE

1.1 Study Title

[^{13}C]-*Spirulina platensis* GEBT – Test-Meal Equivalence Study; Protocol No. PRO-CD-005

1.2 Name of Test Drug/Investigational Product

[^{13}C]-*Spirulina platensis* Gastric Emptying Breath Test (GEBT)

1.3 Indication Studied

Advanced Breath Diagnostics' [^{13}C]-Gastric Emptying Breath Test (i.e., [^{13}C]-GEBT) is indicated for use in the measurement of gastric emptying of solids in humans and as an aid in the initial diagnosis and monitoring of delayed gastric emptying (gastroparesis) in adult patients.

1.4 Study Overview

Forty-four (44) subjects, each of whom was classified as normal with respect to gastric emptying, were planned to be enrolled in this study. Each subject received a GEBT test meal manufactured by the original process (OP) at one test visit, and on a separate occasion each subject was administered a GEBT test meal prepared by the modified process (MP). The order of test meal administration was randomized. Twenty (20) of the 44 subjects received test meals that had been dual-labeled with ^{99m}Tc sulfur colloid, and underwent gastric scintigraphy concurrent with breath testing at each study visit.

1.5 Name of Sponsor

Advanced Breath Diagnostics, LLC (ABD)
105 Westpark Drive, Suite 150
Brentwood, TN 37027
Telephone: 615-376-5464
Fax: 615-376-6384
Primary Contact: Kerry Bush, President

1.6 Protocol Identification

PRO-CD-005-01

The protocol was amended twice during the course of this study, and the amendments were designated PRO-CD-005-02 and -03.

1.7 Development Phase of Study

Study PRO-CD-005 was a Phase 2 trial of test meal equivalence.

The study design was a single-center, open-label, two-sided test of equivalence of normally distributed continuous variables of unknown variance in a group-sequential, two-treatment, two-period cross-over trial design.

1.8 Study Initiation Date

The first subject for Study PRO-CD-005 was enrolled on 01/12/2009.

1.9 Study Completion Date

The final subject visit for Study PRO-CD-005 occurred on 03/01/2010.

1.10 Principal Investigator(s) (PIs)

Lawrence Szarka, M.D.
Assistant Professor of Medicine

Michael Camilleri, M.D.
Professor of Medicine and Physiology

Mayo Clinic
200 First Street SW
Rochester, MN 55905

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1.12 Statement of Compliance

Study PRO-CD-005 was conducted in compliance with good clinical practice (GCP) regulations and guidance, including the archiving of essential documents, as well as with the study protocol and applicable regulations set forth in 21 CFR 812 and 21 CFR 312.

1.13 Date of Report

February 21, 2011

SECTION 2 SYNOPSIS

Name of Sponsor:	Advanced Breath Diagnostics, LLC (ABD)
Name of Finished Product:	^{13}C-Gastric Emptying Breath Test (i.e., ^{13}C-GEBT)
Name of Active Ingredient:	[^{13}C]-*Spirulina platensis*
Protocol Title:	[^{13}C]-*Spirulina platensis* GEBT – Test-Meal Equivalence Study
Protocol Number:	PRO-CD-005
Study Phase:	Phase 2
Study Site:	Mayo Clinic, Rochester MN
Investigators	PI: Lawrence Szarka, M.D. Co-Investigator: Michael Camilleri, M.D.
Study Period:	Date of first enrollment: 01/12/2009 Date of last subject completed: 03/01/2010
Objectives:	To demonstrate by objective evidence that the GEBT test meal produced by a modified manufacturing process (MP) is equivalent to that produced by the original manufacturing process (OP) for use with the gastric scintigraphic and GEBT methods for evaluation of gastric emptying in adults.
Study Design:	A single-center, open-label, two-sided test of equivalence of normally distributed continuous variables of unknown variance in a group-sequential, two-treatment, two-period crossover trial design.
Number of Patients Planned:	Up to 88
Number of Patients Analyzed:	44
Diagnosis and Main Criteria for Inclusion:	Inclusion Criteria: 1. Males and females, 18 – 85 years old. Females of childbearing potential must have negative pregnancy urine test prior to study enrollment and within 48 hours prior to each Gastric Emptying test administration. 2. Ability to eat test meal and provide breath samples. 3. Written informed consent.

Diagnosis and Main Criteria for Inclusion, Continued:	Exclusion Criteria: 1. History or physical exam suggestive of systemic disease such as diabetes mellitus or pathophysiologic disorders such as renal failure, chronic heart disease, chronic respiratory disease, liver disease, or malabsorption syndrome. 2. Symptoms consistent with delayed gastric emptying. 3. History of abdominal surgery except appendectomy. 4. Use of any medications that may alter gastric motility within two days of the study. 5. Use of narcotics or anticholinergics within two days of the study. 6. Females on hormone replacement therapy other than birth control medications. 7. Receipt of an investigational drug within 4 weeks of the study. 8. Pregnancy. 9. Intolerance or allergy to any component of Gastric Emptying test meal (dual-label or GEBT Only) 10. Participant has an identified, clinically significant neurologic or psychiatric disorder(s) that could interfere with compliance to experimental procedures.
Test Product, Dose, Mode of Administration:	The GEBT was conducted as follows. After an overnight fast, the patient consumed the test meal consisting of: ♦ A pasteurized, smoke-flavored, scrambled egg mix containing 43 mg of ^{13}C in approximately 100 mg of *Spirulina platensis* (the diagnostic dosage) ♦ Six (6) Nabisco PREMIUM saltine crackers ♦ Six (6) ounces of potable water The kit also contained breath collection materials, to allow for subsequent analysis of collected breath by Gas Isotope Ratio Mass Spectroscopy (GIRMS). The Dual-label test procedure was conducted with the same test meal; the scintigraphic label, ^{99m}Tc sulfur colloid, was added during test meal preparation. Scintigraphic images were obtained upon completion of the meal and at 45, 90, 120, 150 and 180 minute time points post-ingestion, concurrent with breath sample collection.

Duration of Treatment:	Each subject received one OP meal and one MP meal on two separate occasions, ranging from 48 hours to 3 weeks apart.
Criteria for Evaluation:	For the scintigraphic method, a margin of equivalence for OP vs. MP was set at a half-emptying time ($t_{1/2}$) of 6.6 minutes. After collecting the experimental scintigraphic data, a prospectively defined two-sided equivalence test, applicable to a two-stage group sequential study design, was performed to assess meal equivalence. For the GEBT method, a margin of equivalence for OP vs. MP was set at ± 3.0 kPCD at the most important measurement time points of 90 and 120 minutes. The same two-sided equivalence test used to assess scintigraphic equivalence was utilized to assess meal equivalence using the GEBT experimental data.
Statistical Methods:	The study design utilized a two-sided equivalence testing procedure to test the average difference between results obtained from the Original Production meal (OP) and the Modified Production meal (MP).
Summary – Conclusions:	The study demonstrated by objective evidence that the GEBT test meal produced by the proposed modified manufacturing process (MP) is equivalent to that produced by the original manufacturing process (OP) when used with the gastric scintigraphic and/or the GEBT methods for evaluation of gastric emptying in adults.

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SECTION 4 BACKGROUND INFORMATION

4.1 Regulatory Status of the [^{13}C]-*Spirulina platensis* Gastric Emptying Breath Test

The [^{13}C]-*Spirulina platensis* Gastric Emptying Breath Test (GEBT), which is the subject of this clinical study, is an investigational combination product regulated under both device and drug authorities of the United States of America (US) Food and Drug Administration (FDA). The combination product is classified as a device by the FDA. The clinical trial described in this study report was conducted in the US under Investigational New Drug (IND) application 67,129.

4.2 Proposed Intended Use Statement

Advanced Breath Diagnostics' [^{13}C]-Gastric Emptying Breath Test (i.e., [^{13}C]-GEBT) is indicated for use in the measurement of gastric emptying of solids in humans and as an aid in the initial diagnosis and monitoring of delayed gastric emptying (gastroparesis) in adult patients.

4.3 Description of the [^{13}C]-*Spirulina platensis* GEBT

The GEBT is a non-radioactive stable isotope breath test intended for the measurement of gastric emptying of solids in humans. The proposed commercial breath test kit includes a standardized test meal, which contains the non-radioactive diagnostic drug [^{13}C]-*Spirulina platensis* in smoke-flavored scrambled egg mix. The kit also contains breath collection materials, to allow for subsequent analysis of collected breath by Gas Isotope Ratio Mass Spectroscopy (GIRMS).

The GEBT is conducted as follows:

After an overnight fast, the patient consumes the test meal consisting of:

- A pasteurized, smoke-flavored, scrambled egg mix containing 43 mg of ^{13}C in approximately 100 mg of *Spirulina platensis*. The egg/*Spirulina platensis* mixture proposed for commercialization is provided as a freeze-dried powder for rehydration and cooking prior to consumption by the patient.
- Six (6) Nabisco PREMIUM saltine crackers
- Six (6) ounces of potable water

Following ingestion, the test meal is triturated by the stomach and then passes through the pylorus into the intestine where the [^{13}C]-*Spirulina platensis* is digested, absorbed, and metabolized, giving rise to ^{13}C-labeled carbon dioxide ($^{13}CO_2$) expired in the breath. Breath samples, collected before and after ingestion of the test meal, are sent to a central laboratory for analysis by GIRMS to determine the ratio of $^{13}CO_2/^{12}CO_2$. This ratio is used to calculate the $^{13}CO_2$ excretion rate. By measuring the change in excretion over time, the rate of gastric emptying can be determined.

4.4 Description of the Diagnostic Drug Component, [^{13}C]-*Spirulina platensis*

Spirulina species including *Spirulina platensis* are blue-green microalgae with a history of use in the human diet as a source of protein.[1] Spirulina was acknowledged by the US FDA as a legally marketed food in 1981[2], and is currently consumed in the US as a dietary supplement at doses of 3 to 4.5 grams per day. The safety of Spirulina has been established through centuries of use as a food and numerous toxicology studies. Acute, subchronic, and chronic toxicology, teratogenicity and mutagenicity studies have shown no toxic effects.[3], [4], [5], [6], [7]

[^{13}C]-*Spirulina platensis* for use in this test is produced by culturing a pure axenic inoculum of *Spirulina platensis* in a growth medium enriched in ^{13}C, a non-radioactive stable isotope of carbon. ^{13}C stable isotope labeling is inherently safe as 1.1% of all carbon in our bodies and in the food we eat is ^{13}C.

4.5 Proof of Principle Studies

Two ABD-sponsored clinical trials were conducted at the Mayo Clinic between 1999 and 2001, which demonstrated proof of principle of the GEBT. A total of 57 healthy subjects were studied, 24 of which were pharmacologically induced to either accelerated (via erythromycin i.v.) or delayed (via atropine i.v.) gastric emptying.[8], [9] Gastric emptying was measured following a test meal comprising two (2) scrambled eggs, a slice of wheat toast and eight (8) ounces of skim milk. The eggs were double labeled with 0.5 mCi ^{99m}Tc sulfur colloid and 200 mg of [^{13}C]-*Spirulina platensis* containing 80 mg of ^{13}C. Scintigraphic images and breath samples were obtained at periodic intervals before and after test meal consumption in order to measure gastric emptying. A generalized linear regression model was developed to correlate the scintigraphically-derived gastric half-emptying time ($t_{1/2}$) to the $^{13}CO_2$ excretion rates. The model was internally validated by the leave-one-out method to generate the GEBT $t_{1/2}$ results.

The mean difference in $t_{1/2}$ between the two methods was 0.15 minutes with standard deviation of 35.5 minutes. The GEBT correctly identified the presence or absence of gastroparesis (as defined by a scintigraphic $t_{1/2} > 150$ minutes) in 56 of 57 cases.

There were no adverse events associated with the investigational device in either of these studies. These studies were published.[8], [9]

4.6 Summary of the Overall Investigational Plan to Validate the Advanced Breath Diagnostics' GEBT

The Investigational Plan for Advanced Breath Diagnostics' (ABD's) GEBT consists of an ordered sequence of clinical studies, each with its own specific objectives, as summarized in Table 1.

Table 1. Investigational Plan to Validate the Gastric Emptying Breath Test (GEBT)

Study Name and Protocol Number	Study Objective(s)	Study Site(s)
1. GEBT Dose Finding Study, PRO-CD-001	Selection of a safe and effective [^{13}C]-*S. platensis* dose and test meal composition for use in further studies	1. Northwestern (Chicago, IL) 2. Vanderbilt (Nashville, TN)
2. GEBT Biologic Variation / Reference Range Study, PRO-CD-002	1. Estimation of time-related, within-subject total variability (imprecision) with GEBT 2. Estimation of the GEBT Reference Range for normal adults	1. Northwestern (Chicago, IL) 2. Vanderbilt (Nashville, TN)
3. GEBT Dual-Label* Calibration Trial, PRO-CD-003	1. Estimation of the Reference Range for scintigraphic results using the GEBT test meal 2. Add to GEBT Reference Range data for normal adults 3. Estimation of total variability (imprecision) of scintigraphy and GEBT measurements on normal and marginal† gastroparetic test subjects	Mayo Clinic (Rochester, MN)
4. GEBT Dual-Label* Validation Trial, PRO-CD-004	Validation of the GEBT for use in diagnosis and monitoring of delayed gastric emptying	Mayo Clinic (Rochester, MN)
5. Test Meal Equivalence Study, PRO-CD-005	Demonstrate that the GEBT test meal produced by a modified manufacturing process (MP) is equivalent to that produced by the original manufacturing process (OP)	Mayo Clinic (Rochester, MN)

* The term "dual-label" refers to a GEBT test meal that contains both ^{13}C-Spirulina and ^{99m}Tc sulphur colloid, the labeling substance used in gastric scintigraphy. After consuming the test meal, gastric emptying testing of the subject is conducted simultaneously by scintigraphy and by GEBT.
† "Marginal gastroparetics" are defined as those with gastric emptying metrics ranging from slow (low) normal to mild gastroparesis.

The first three studies, PRO-CD-001, PRO-CD-002 and PRO-CD-003 were Phase 2 exploratory trials. Study PRO-CD-004 was the confirmatory (pivotal) trial, in which the overall diagnostic concordance, sensitivity and specificity of the GEBT were compared with scintigraphic diagnoses. Shortly after completion of the confirmatory trial (PRO-CD-004), a key contract manufacturer became unavailable to produce the lyophilized egg/^{13}C-Spirulina test meal used in the GEBT. An alternative contract manufacturer was selected to produce the GEBT test meal, and PRO-CD-005 (the Test Meal Equivalence Trial) was added to the original investigational plan to demonstrate functional equivalence of the test meals.

Briefly, with regard to each of the five studies summarized in Table 1:

- The results from Study PRO-CD-001, the GEBT dose-finding study, established the composition of the GEBT test meal and dose of carbon-13 for use in all subsequent studies.
- The results of Studies PRO-CD-002 and PRO-CD-003 were used to establish diagnostic cut-off points (COPs) for both the GEBT and scintigraphic methods in order to convert continuous metric results obtained with each respective method into diagnostic classifications.
- Study PRO-CD-004 was the confirmatory (pivotal) trial, in which the overall diagnostic agreement between the GEBT and gastric scintigraphy was demonstrated.
- Study PRO-CD-005 compared test results and demonstrated equivalence between the test meal used in Study PRO-CD-004 and the test meal intended for commercialization. Equivalence was demonstrated by both GEBT and gastric scintigraphy.

Study PRO-CD-005 is the subject of this report.

SECTION 5 LIST OF ABBREVIATIONS AND DEFINITION OF GEBT METRICS

5.1 Abbreviations

ABD: Advanced Breath Diagnostics, LLC

BMR: Basal Metabolic Rate

^{13}C-*Sp.*: [^{13}C]-*Spirulina platensis*

COP: Cut-Off Point

CO_2PR: CO_2 Production Rate

CRF: Case Report Form

DOB: Delta over Baseline

GEBT: Gastric Emptying Breath Test (using ^{13}C-*Spirulina)*

GIRMS: Gas Isotope Ratio Mass Spectrometry

ICF: Informed Consent Form

kPCD: 1000 x PCD

MP: Modified Process

OP: Original Process

PCD: Percent Dose

PDB: Pee Dee Belemnite, i.e., a ^{13}C international reference standard

PI: Principal Investigator

$Prop_t$: The proportion of scintigraphic tracer emptied from the stomach at time t.

t: Time t - a breath collection or scintigraphic scanning time point

$t_{1/2}$: Scintigraphic half emptying time of the standardized GEBT test meal

5.2 Definition of GEBT and Scintigraphic Metrics

5.2.1 GEBT Metric

The generally preferred GEBT metric, and that used in Study PRO-CD-005, is the Percent Dose (PCD) excreted at time t after consumption of the test meal. To provide a more convenient scale, PCD is multiplied by 1000 to produce kPCD at any time, t.

$$kPCD_t = \left[\frac{DOB * CO_2PR * R_s * 13}{10 * dose}\right] * 1000$$

Where:

DOB = The measured difference in the ratio [$^{13}CO_2/^{12}CO_2$] between a post-meal breath specimen at any time (t-minutes) and the baseline breath specimen.

CO_2PR = CO_2 Production Rate (mmol CO_2/min) calculated using the Schofield equations[10] which incorporate the patient's age, gender, height and weight.

R_s = The ratio [$^{13}CO_2/^{12}CO_2$] in the reference standard (Pee Dee belemnite) for these measurements. $R_s = 0.0112372$

13 = the atomic weight of Carbon-13

10 = A constant factor for converting units

dose = the weight (mg) of Carbon-13 in the dose of [^{13}C]-*S. platensis* administered to the patient in the test meal. Since [^{13}C]-*Spirulina platensis* is approximately 43% Carbon-13, a 100 mg dose of [^{13}C]-*Spirulina platensis* contains approximately 43 mg of Carbon-13.

Subject test results, GEBT reference ranges and GEBT reference range cut-off points (COPs) are all expressed in the kPCD metric.

See Section 9.4.1 for a complete, detailed description of GEBT metric mathematics.

5.2.2 Scintigraphic Metric

For each gamma scintigraphy scan performed, a region of interest (ROI) is drawn around the stomach on the anterior and posterior images for each time frame. Data are corrected for decay of ^{99m}Tc sulphur colloid, the labeling substance used in scintigraphy. To correct for depth or tissue attenuation, the counts of each anterior and posterior ROI are multiplied together, and the square root of the product is taken to obtain the geometric mean. The scintigraphic gastric emptying (GE) metric, $Prop_t$, is the proportion of tracer emptied from the stomach at time, t. With a power exponential model, these data are also used to calculate the GE half-time ($t_{1/2}$) after estimating the constants κ and β in the power exponential model, $Prop_t = \exp(-\kappa t^{\beta})$.

SECTION 6 ETHICS

6.1 Institutional Review Boards

The Institutional Review Board at Mayo Clinic, Rochester, MN (IRB; see Appendix 15.1, Section 15.1.4) reviewed and approved all versions of the protocol and the informed consent forms (Appendix 15.1, Sections 15.1.1 and 15.1.5, respectively).

6.2 Ethical Conduct of the Study

The site was monitored on an ongoing basis to ensure that subjects enrolled met study requirements and that Good Clinical Practice (GCP) regulations and guidance were being followed. The monitor was required to report promptly to the Sponsor any data that were suggestive of non-adherence to the protocol, to facilitate prompt intervention with the site.

The study was conducted in accordance with ethical standards according to the Declaration of Helsinki. Premature termination of this study could occur at the discretion of the IRB. The Sponsor, Advanced Breath Diagnostics, LLC, also retained the right to discontinue this study at any time.

6.3 Patient Information and Consent

Each subject was required to read and sign the Informed Consent Form (ICF) prior to enrollment into the study and before any study procedures were performed. Subjects were informed that they could withdraw from the study at any time for any reason.

SECTION 7 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

7.1 Principal Investigators

This study was conducted at the Mayo Clinic, Rochester, MN with one principal investigator (PI) and one co-investigator. The affiliation and curriculum vitae for both are provided in Appendix 15.1, Section 15.1.6.

7.2 Administrative Structure

Advanced Breath Diagnostics, LLC (ABD) was the Sponsor of this study. The following is a list of organizations and individuals involved in the conduct and reporting of the study.

Statistics	Robert F. Martin, Ph.D. Director of Regulatory and Clinical Affairs Advanced Breath Diagnostics, LLC 105 Westpark Drive, Suite 150 Brentwood, TN 37027 Telephone: (615) 376-5464 Fax: (615) 376-6384
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SECTION 8 STUDY OBJECTIVES

8.1 Study Objectives

The intent of the study was to demonstrate by objective evidence that the GEBT test meal produced by a proposed modified manufacturing process (MP) was equivalent to that produced by the original manufacturing process (OP) for use with the gastric scintigraphic and GEBT methods for evaluation of gastric emptying in adults.
Note: The unit-dose GEBT test meal (the drug product) manufacturing process has two major processes: (1) manufacture of the drug substance, [^{13}C]-*Spirulina platensis*, and (2) manufacture of the unit-dose pouch which contains a uniform blend of specific amounts of drug substance and dried, formulated, pasteurized egg mix.

For the present study, Original Process (OP) and Modified Process (MP) are defined as follows:

Original Process:	The combination of processes used to manufacture the drug substance and drug product in the test meals used in Parts B and C of Study PRO-CD-002, the GEBT Calibration Study (PRO-CD-003) and the Validation Study (PRO-CD-004).
Modified Process:	The combination of processes used to manufacture the drug substance and drug product (test meal) with new vendors, methodologies and equipment. The modified process is proposed for manufacture of commercial product.

8.2 Necessity of Study – Unanticipated Change in Test Meal Manufacturer

A key contract manufacturer, Oregon Freeze Dry (OFD, Albany, OR), informed ABD in late 2008 that it would no longer provide comprehensive manufacturing services for the production of the lyophilized egg/^{13}C-Spirulina test meal used in ABD's Gastric Emptying Breath Test (GEBT). OFD had manufactured the GEBT test meal used in Studies PRO-CD-001 through PRO-CD-004 of ABD's Investigational Plan (see Table 1).

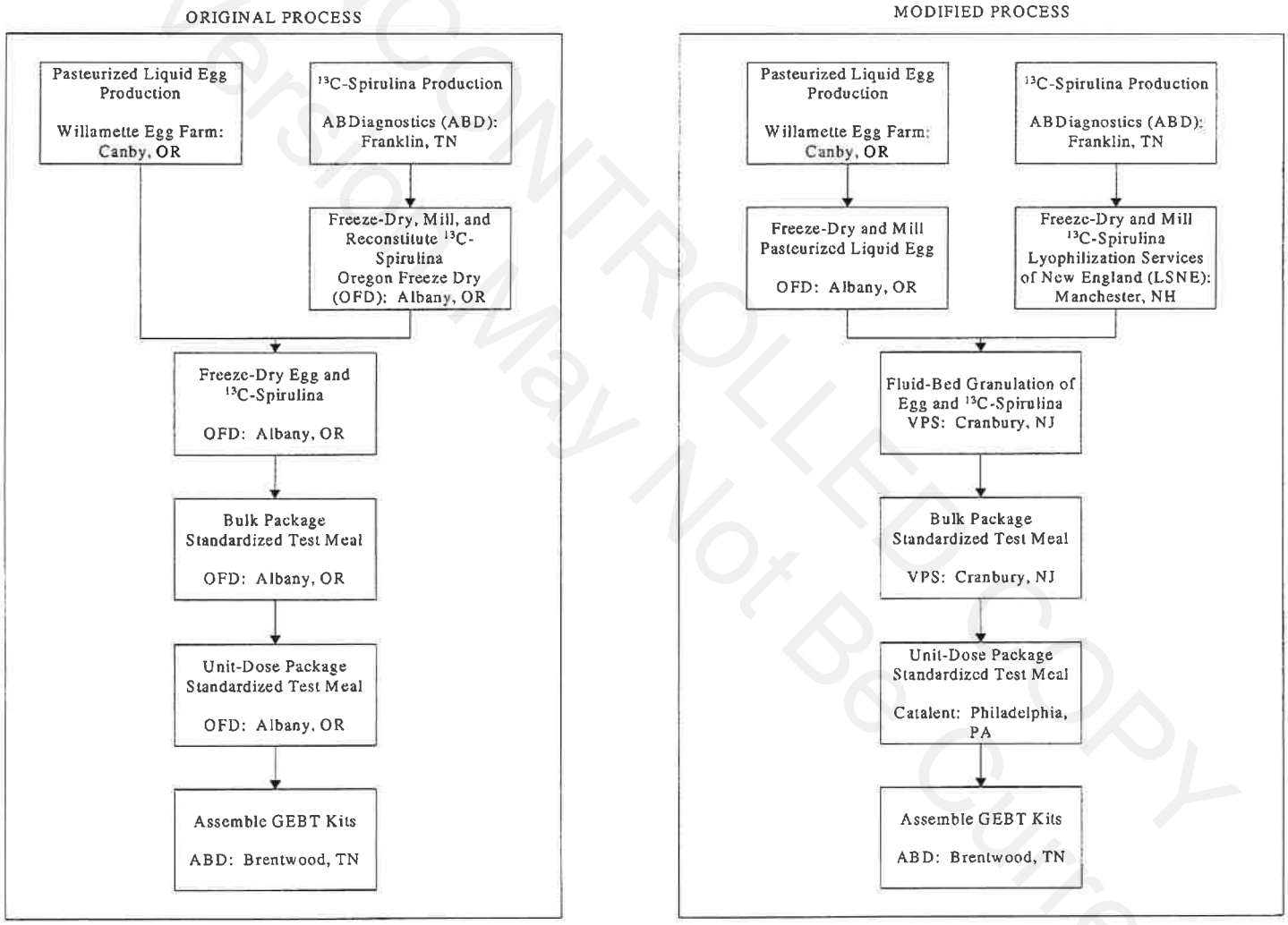
Based on this notification from OFD, ABD evaluated alternative contract manufacturers to produce the GEBT test meal. Lyophilization Services of New England (LSNE), Manchester, NH, was selected to lyophilize the [^{13}C]-*Spirulina platensis* drug substance produced by ABD in Brentwood, TN. CMIC-VPS, Cranbury, NJ was selected to combine the lyophilized [^{13}C]-*Spirulina platensis* drug substance with the scrambled egg mix via fluid-bed granulation to produce bulk drug product. Catalent Pharma Solutions, Philadelphia, PA, was selected to unit-dose pouch the bulk drug product into individual GEBT test meals.

Following process qualification at the new manufacturing sites, in vitro laboratory test results for product manufactured by the modified process (MP) intended for commercial product conformed to specifications established for and applied to product manufactured

by the original process (OP). Study PRO-CD-005 was then designed and performed to determine if the manufacturing process changes significantly affected the physiologic (in vivo) performance of the GEBT test meal. This study utilized MP GEBT test meal Lot D008-004, which was produced according to current Good Manufacturing Practice (cGMP) regulations.

Figure 1 provides flow charts of the original OFD manufacturing processes and the modified manufacturing processes, including identification of the subcontractor selected to perform each process. In the flow charts, process steps with white backgrounds are identical for the original and modified processes. Process steps with gray backgrounds differ from the original process in manufacturing site and/or manufacturing process. Note that the raw materials for the test meal (pasteurized liquid egg and [^{13}C]-*Spirulina platensis*) continue to be produced by the original manufacturers and processes and must meet the same specifications established for OP product. The primary change to the processes is the substitution of fluid-bed granulation, performed by CMIC-VPS, for lyophilization at OFD in the production of the bulk drug product (test meal). The test meal ingredients and dose of carbon-13 label (supplied by ^{13}C-Spirulina) remain unchanged.

Figure 1. Original vs. Modified Test Meal Production Process



SECTION 9 INVESTIGATIONAL PLAN

9.1 Study Design

Study PRO-CD-005, conducted at Mayo Clinic's General Clinical Research Center (GCRC), Rochester, MN, was a prospective, open-label, two-sided test of equivalence of normally distributed continuous variables of unknown variance in a group-sequential, two-treatment, two-stage crossover trial.[11] Note: the two test procedures (GEBT and scintigraphy) had different sample size requirements in the group sequential design. Additionally, interim assessment of equivalence at Stage 1 was allowed after half of the required number of study subjects had been tested (20 by scintigraphy and 44 by GEBT). Figure 2 depicts the process of randomized testing utilized to accommodate all potential testing required in order to make a valid assessment of equivalence by each testing method. Specific randomized test sequences for each of the five possible sequences referenced in Figure 2 are displayed in Table 2.

Up to 88 test subjects, each of whom was classified by the Inclusion/Exclusion criteria as normal with respect to gastric emptying, were to be included in the study. Following screening, subjects who qualified for enrollment received the GEBT on two separate occasions, ranging from 48 hours to 3 weeks apart. Each subject received one OP test meal and one MP test meal, according to the randomization scheme displayed in Table 2. Additionally, as the study was designed to assess the equivalence of OP and MP test meals by both the scintigraphic and GEBT methods, a subset of subjects received dual-labeled test meals at both administrations, also according to the randomization scheme in Table 2. The following paragraphs describe the activities that took place at each study visit.

Visit 1: The subject reported to the test site for a screening visit. Following provision of informed consent by the subject, a medical history was taken, including concomitant medications, and a physical exam was conducted. Women of child-bearing potential were required to take a urine pregnancy test.

Visits 2 and 3: The subject reported to the test site at approximately 8:00 AM after an overnight fast, at which time either an OP or MP gastric emptying test was performed, according to the randomization scheme displayed in Table 2. Baseline breath samples were collected, after which the test meal containing [^{13}C]-*Spirulina platensis* was consumed. Breath samples were collected at 45, 90, 120, 150, and 180 minute time points, measured from the end of test meal ingestion. For subjects who received dual-labeled test meals (see Table 2), each meal also contained 0.5 mCi ^{99m}Tc sulphur colloid. Scintigraphic images were acquired upon completion of the meal and at 45, 90, 120, 150, and 180 minute time points, on the same schedule as collection of breath samples. Two minute anterior and posterior planar images were acquired with a General Electric (GE) 500 gamma camera with circular LFOV, using a medium energy collimator, operated through a MEDX Nuquest workstation. Additional details of how the test meal was

prepared and administered, and how breath samples were collected, are provided in Appendix 15.1, Section 15.1.2.

Breath samples collected from each subject were shipped to Advanced Breath Diagnostics, LLC, where they were assayed to determine the ratio of ^{13}C to ^{12}C by GIRMS. These results were used to calculate the kPCD and other parameters, as described in Section 9.4.1 of this report. Scintigraphic images were processed at the Mayo Clinic, Rochester, MN. The fraction of test meal emptied from the stomach was determined as described in Section 5.2 of this report.

Figure 2. Randomization of Test Sequences in the Group Sequential Design

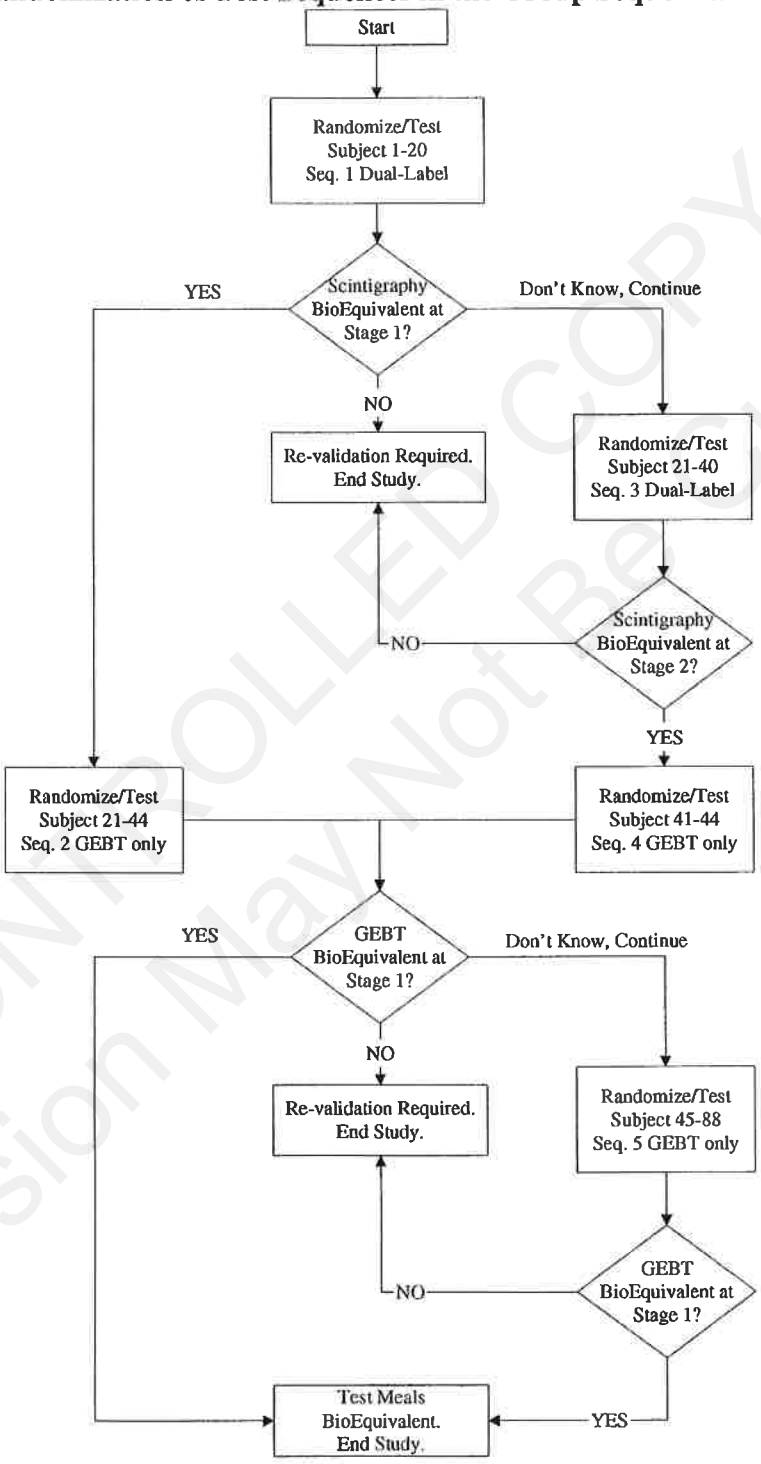


Table 2. Planned Randomized Test Sequences

Sequence #1 (Dual-Label)		Sequence #2 (GEBT Only)		Sequence #3 (Dual-Label)		Sequence #4 (GEBT Only)		Sequence #5 (GEBT Only)	
TS #	Order	TS #	Order	TS #	Order	TS #	Order	TS #	Order
1	OP/MP	21	OP/MP	21	MP/OP	41	MP/OP	45	OP/MP
2	OP/MP	22	MP/OP	22	MP/OP	42	OP/MP	46	MP/OP
3	MP/OP	23	OP/MP	23	OP/MP	43	OP/MP	47	MP/OP
4	OP/MP	24	OP/MP	24	OP/MP	44	MP/OP	48	MP/OP
5	MP/OP	25	MP/OP	25	MP/OP			49	OP/MP
6	MP/OP	26	MP/OP	26	OP/MP			50	MP/OP
7	OP/MP	27	OP/MP	27	MP/OP			51	OP/MP
8	OP/MP	28	MP/OP	28	OP/MP			52	OP/MP
9	MP/OP	29	OP/MP	29	OP/MP			53	OP/MP
10	MP/OP	30	MP/OP	30	MP/OP			54	MP/OP
11	MP/OP	31	OP/MP	31	OP/MP			55	OP/MP
12	OP/MP	32	OP/MP	32	MP/OP			56	OP/MP
13	OP/MP	33	OP/MP	33	OP/MP			57	MP/OP
14	MP/OP	34	OP/MP	34	MP/OP			58	OP/MP
15	MP/OP	35	OP/MP	35	MP/OP			59	MP/OP
16	OP/MP	36	MP/OP	36	OP/MP			60	MP/OP
17	OP/MP	37	MP/OP	37	OP/MP			61	OP/MP
18	OP/MP	38	OP/MP	38	MP/OP			62	OP/MP
19	MP/OP	39	MP/OP	39	OP/MP			63	OP/MP
20	MP/OP	40	OP/MP	40	MP/OP			64	OP/MP
		41	MP/OP					65	MP/OP
		42	MP/OP					66	MP/OP
		43	MP/OP					67	OP/MP
		44	MP/OP					68	MP/OP
								69	MP/OP
								70	OP/MP
								71	MP/OP
								72	OP/MP
								73	MP/OP
								74	OP/MP
								75	MP/OP
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								79	OP/MP
								80	MP/OP
								81	MP/OP
								82	MP/OP
								83	OP/MP
								84	OP/MP
								85	OP/MP
								86	OP/MP
								87	MP/OP
								88	MP/OP

Statistical testing to assess equivalence was prospectively defined. A two-sided test of equivalence was constructed; the conditions to be satisfied to declare equivalence are fully described in Protocol PRO-CD-005-03, section 5(A.), page 13; see Appendix 15.1, Section 15.1.1 of this study report. Assessment of equivalence was allowed after Stage 1 of the 2-stage sequential design was completed, as outlined in Figure 2. Tables 3 and 4 display the number of subjects required to be tested by each method in each stage of the present study.

Table 3. Enrollment Plan for Gastric Scintigraphy

Stage	Nx_k *	Ny_k **	Total Subjects	Total Scintigraphic Results
1	10	10	20	40
2 (if necessary)	10	10	20	40
Totals	20	20	40	80

* Nx_k is the number of subjects who will receive the MP meal first.
** Ny_k is the number of subjects who will receive the OP meal first.

Table 4. Enrollment Plan for the GEBT

Stage	Nx_k *	Ny_k **	Total Subjects	Total GEBT Results
1	22	22	44	88
2 (if necessary)	22	22	44	88
Totals	44	44	88	176

* Nx_k is the number of subjects who will receive the MP meal first.
** Ny_k is the number of subjects who will receive the OP meal first.

9.1.1 Initial Discontinuation of Study PRO-CD-005 and Study Re-start

The objective of original study protocol PRO-CD-005-01, "[^{13}C]-*Spirulina platensis* GEBT – Test Meal Equivalence Study," was to demonstrate that the GEBT test meal produced by a modified manufacturing process (MP) was equivalent to the test meal produced by the original manufacturing process (OP) for use with gastric scintigraphic and GEBT methods for evaluation of gastric emptying in adults. However, Study PRO-CD-005 was discontinued after 14 subjects had completed testing with dual-labeled OP and MP test meals due to early findings that GEBT results (kPCD values) obtained with the MP test meals were significantly smaller than results from the OP test meals.

Table 5 displays the discrepancy observed in average kPCD values between OP and MP at the most important GEBT measurement time points after initiation of the equivalence study.

Table 5. Discrepancy Between Average OP and MP kPCD Values for First 14 Subjects

Test Meal	N	Mean kPCD Values		
		90 Minutes	120 Minutes	150 Minutes
OP	14	48.6	58.1	60.1
MP	14	38.0	46.8	49.1
MP - OP		-10.6	-11.3	-11.0

After qualification of the modified drug product (^{13}C-Spirulina/egg mix) production process, three minor adjustments to the ^{13}C-Spirulina (drug substance) algal growth process were assessed to determine if they would provide improvements in yield and/or process efficiency, and were incorporated into the manufacture of ^{13}C-Spirulina used in the MP test meals administered to the first 14 subjects in this study. These three adjustments are listed below. All growth process parameters other than those noted below remained the same as in the original process.

1. At the 20 L bioreactor stage of growth in the original process, cultures are continuously mixed via rotation on a platform rotating at ~80 rpm. In the "modified process," additional gentle stirring, via stir bar, was added.

2. At the end of the 20 L growth stage in the original process, the algal contents of the bioreactors are harvested via continuous-flow centrifugation. Because algal cultures quickly settle out after rotation and mixing are stopped, centrifugation was omitted in the modified process. Instead, supernatant was removed once the cultures settled and the residual algal mass was collected.

3. In the original process, the algal mass is washed three times with room temperature sterile deionized (DI) water to remove residual salts and growth media elements. In the modified process, cold water was utilized and one additional wash was conducted.

After thorough investigation, ABD determined that the difference in MP and OP test meal diagnostic performance was due to the incorporation of these process modifications. Although [^{13}C]-*Spirulina platensis* batches produced in this manner met all drug substance release test specifications, in vitro testing did not adequately predict in vivo performance.

In order to resolve this issue, [^{13}C]-*Spirulina platensis* drug substance Lot API003-007 was produced in accordance with the original growth process used to produce the ^{13}C-Spirulina that was incorporated into all OP test meals (this original manufacturing process is also the intended commercial manufacturing process). Lot API003-007 was then incorporated into MP test meal Lot D008-004, which was used in the Study PRO-CD-005 Re-Start. Hence, the OP and MP meals utilized in the Study PRO-CD-0-05 Re-Start contain identically produced but independent lots of [^{13}C]-*Spirulina platensis* Drug Substance.

Study PRO-CD-005 was re-started under revised protocol PRO-CD-005-02. The amended protocol differed from the original only in that the test meal administration sequence was amended to accommodate re-starting the study and maintaining test meal randomization.

Upon review of data from the first 14 subjects who received dual-labeled test meals in Study PRO-CD-005, it was concluded that, although the GEBT results were not acceptable, results collected for these test subjects provided valid scintigraphic comparisons between the OP and MP test meals, making it unnecessary to repeat scintigraphic testing on the first 14 subjects in the Study PRO-CD-005 Re-start. This conclusion was based on the following observations.

1. Gastric scintigraphy is a direct physical measurement of gastric emptying. The weight, meal matrix and caloric value of the MP and OP meals were identical.

2. Although the average GEBT kPCD values between OP and MP were significantly different, Table 6 demonstrates that the average scintigraphic $t_{½}$ values were very close between OP and MP meals in the first 14 subjects tested. Note: Table 6 also displays the average scintigraphic $t_{½}$ value for 30 subjects administered an OP meal in Study PRO-CD-003; this result was also very comparable to the average scintigraphic $t_{½}$ value observed with the MP test meal.

Table 6. OP and MP $t_{½}$ Values: First 14 Subjects in Study PRO-CD-005

Scintigraphy	Reference Range Study PRO-CD-003 N=30	Meal Equivalence Study PRO-CD-005 N=14	
Test Meal	**OP**	**OP**	**MP**
Mean $t_{½}$ (minutes)	66.0	67.6	65.0
Standard Deviation	11.8	14.0	14.3

3. Statistical Considerations: Based on an interim analysis of scintigraphic data collected from the first 14 dual-label test subjects, the test statistics presented in Table 7 were calculated according to definitions prescribed in Section 5.F of the protocol (see Appendix 15.1, Section 15.1.1).

Table 7. Study PRO-CD-005 Calculated Test Statistics

Number of Results	Acceptance/Rejection Test Limits	Observed Test Statistics
$Nx_k = 8$	Reject Equivalence Limit = 0.720	$(T^+)_{observed} = -3.169$
$Ny_k = 6$	Accept Equivalence Limit = -1.424	$(T^-)_{observed} = 1.126$
		$\Theta(t_{1/2})_{observed} = -3.14$ min

If the first 14 subjects constituted Stage 1 of the group sequential two-stage trial, the calculations call for the following actions:

1. Do not reject scintigraphic equivalence at this stage.
2. Do not accept scintigraphic equivalence at this stage but continue to collect data.

4. Safety Considerations: The labeling substance used for scintigraphy, ^{99m}Tc sulphur colloid, is a radioactive tracer. Unnecessary exposure of healthy test subjects to radioactivity is ill-advised.

In view of these considerations, Sequence 1 was revised so that the scintigraphic results collected for 14 test subjects in the initial discontinued study could be used in the final assessment of scintigraphic test meal equivalence. As presented in Table 8, revised Sequence 1 was subdivided into 2 parts. Sequence 1a defined the test meal randomization scheme for replacing the 14 GEBT only subjects; for these 14 subjects GEBT-only tests (no dual-label tests) were performed. Sequence 1b defined the test meal randomization scheme for 6 additional dual-label test subjects to be enrolled in order to satisfy the Stage 1 requirement of 20 dual-label test subjects. Table 8 displays both original Sequence 1 and amended Sequences 1a and 1b.

Scintigraphic test results collected from the 14 patients in original Sequence 1 were combined with scintigraphic results from the 6 additional patients completed in Sequence 1b (N=20). Stage 1 calculations to assess scintigraphic equivalence were then performed as described in section 5(F) of the study protocol.

Table 8. Original Study PRO-CD-005 Test Sequence 1 and Revised PRO-CD-005 Re-start Test Sequences 1a and 1b

Original (PRO-CD-005-01)	
Sequence #1 (Dual-Label)	
TS #	Order
1	OP/MP
2	OP/MP
3	MP/OP
4	OP/MP
5	MP/OP
6	MP/OP
7	OP/MP
8	OP/MP
9	MP/OP
10	MP/OP
11	MP/OP
12	OP/MP
13	OP/MP
14	MP/OP
15	MP/OP
16	OP/MP
17	OP/MP
18	OP/MP
19	MP/OP
20	MP/OP

Amended (PRO-CD-005-02)	
Sequence #1a (GEBT Only)	
TS #	Order
1	OP/MP
2	OP/MP
3	MP/OP
4	OP/MP
5	MP/OP
6	MP/OP
7	OP/MP
8	OP/MP
9	MP/OP
10	MP/OP
11	MP/OP
12	MP/OP
13	OP/MP
14	MP/OP
Sequence #1b (Dual-Label)	
15	MP/OP
16	OP/MP
17	OP/MP
18	OP/MP
19	MP/OP
20	OP/MP

9.2 Selection of Study Population

Up to 88 apparently normal and healthy volunteers, male and female, 18 to 85 years of age could be enrolled in the study. Volunteers were to be recruited by public advertisement at the Mayo Clinic.

9.2.1 Inclusion Criteria

1. Males and females, 18 – 85 years old. Females of childbearing potential must have negative pregnancy urine test prior to study enrollment and within 48 hours prior to each Gastric Emptying test administration.
2. Ability to eat test meal and provide breath samples.
3. Written informed consent.

9.2.2 Exclusion Criteria

1. History or physical exam suggestive of systemic disease such as diabetes mellitus or pathophysiologic disorders such as renal failure, chronic heart disease, chronic respiratory disease, liver disease, or malabsorption syndrome.
2. Symptoms consistent with delayed gastric emptying.
3. History of abdominal surgery except appendectomy.
4. Use of any medications that may alter gastric motility within two days of the study.
5. Use of narcotics or anticholinergics within two days of the study.
6. Females on hormone replacement therapy other than birth control medications.
7. Receipt of an investigational drug within 4 weeks of the study.
8. Pregnancy.
9. Intolerance or allergy to any component of Gastric Emptying test meal (Dual-Label or GEBT Only).
10. Participant has an identified, clinically significant neurologic or psychiatric disorder(s) that could interfere with compliance to experimental procedures.

9.3 [^{13}C]-*Spirulina platensis* GEBT

The GEBT is a non-radioactive stable isotope breath test intended for the measurement of gastric emptying of solids in humans. The proposed commercial breath test kit includes a standardized test meal, which contains the non-radioactive diagnostic drug [^{13}C]-*Spirulina platensis* in smoke-flavored scrambled egg mix. The kit also contains breath collection materials, so that collected breath can be subsequently analyzed by GIRMS.

The GEBT was conducted in this study as follows.

After an overnight fast, the patient consumed the test meal consisting of:

- A pasteurized, smoke-flavored, scrambled egg mix containing 43 mg of ^{13}C in approximately 100 mg of *Spirulina platensis*
- Six (6) Nabisco PREMIUM saltine crackers
- Six (6) ounces of potable water

Following ingestion, the test meal is triturated by the stomach and then passes through the pylorus into the intestine where the [^{13}C]-*Spirulina platensis* is digested, absorbed, and metabolized, giving rise to $^{13}CO_2$ expired in the breath. Breath samples, collected before and after ingestion of the test meal, are sent to a central laboratory for analysis by GIRMS to determine the ratio of $^{13}CO_2/^{12}CO_2$. This ratio is used to calculate the $^{13}CO_2$ excretion rate. By measuring the change in excretion over time, the rate of gastric emptying can be determined.

The dual-label test procedure utilized the standard GEBT test as described above with the addition of 0.5 mCi ^{99m}Tc sulphur colloid. Testing was then conducted as follows:

After an overnight fast, the patient consumed the test meal consisting of:

- A pasteurized, smoke-flavored, scrambled egg mix containing 43 mg of ^{13}C in approximately 100 mg of *Spirulina platensis* and 0.5 mCi ^{99m}Tc sulphur colloid
- Six (6) Nabisco PREMIUM saltine crackers
- Six (6) ounces of potable water

Scintigraphic scanning with anterior and posterior cameras was performed with the patient standing. Two minute anterior and posterior planar images were acquired using a General Electric (GE) 500 gamma camera with circular LFOV, which utilizes a medium energy collimator, and is operated through a MEDX Nuquest workstation. Imaging started upon completion (consumption) of the dual-label test meal and scans were then obtained at 45, 90, 120, 150, and 180 minute time points. Breath samples were taken at baseline (before the test meal was consumed) and thereafter on the same time schedule as the scintigraphic scanning procedure.

9.3.1 [^{13}C]-*Spirulina platensis* GEBT Tests Administered

The GEBT administered to each subject in this study consisted of the following.

- Approximately 100 mg lyophilized ^{13}C-Spirulina, contributing 43 mg of exogenous ^{13}C, in 27 grams of lyophilized egg mix, which is rehydrated and cooked at the clinical site
- Six (6) Nabisco PREMIUM saltine crackers
- 6 ounces of potable water for consumption with the meal

For 20 of the subjects enrolled in this study, the GEBT test meal was a dual label test meal, containing 0.5 mCi ^{99m}Tc sulphur colloid. The sulfur colloid was added prior to cooking the meal for ingestion. The test meal kits and the other study supplies were stored at room temperature in a secured locked area until dispensed. The subject initials were verified with each subject prior to administering the GEBT test meal. Subjects were instructed to eat the meal within 10 minutes.

9.3.2 Identity of the Investigational Product

9.3.2.1 Identity of Investigational Product Used in Initial, Discontinued Study PRO-CD-005

9.3.2.1.1 Preparation of Original Process (OP) Meal and GEBT Kits

Advanced Breath Diagnostics LLC (ABD) manufactured bulk [^{13}C]-*Spirulina platensis* Lot API003-002, used in the OP meals administered in the present study, according to the

intended commercial process at its Drug Substance production facility in Franklin, TN. Bulk ^{13}C-Spirulina Lot API003-002 was incorporated into pasteurized formulated liquid egg (prepared by Willamette Egg Farms, Canby, OR), mixed and subsequently lyophilized to yield bulk drug product at Oregon Freeze Dry (OFD), Albany, OR. Twenty-seven (27) gram aliquots of the bulk drug product were subsequently unit-dose packaged (also at OFD) into foil pouches to yield finished Drug Product Lot D007-001.

Individual sealed pouches from Drug Product Lot D007-001 were inserted into OP GEBT test kits during kit assembly at ABD, Brentwood, TN. Sixty-seven (67) OP GEBT kits from kit manufacturing Lot K009-006 were shipped to the study site (Mayo Clinic, Rochester MN).

9.3.2.1.2 Preparation of Modified Process (MP) Meal and GEBT Kits

The [^{13}C]-*Spirulina platensis* used in the MP meals administered in the initial part of this study was manufactured by Advanced Breath Diagnostics, LLC (ABD) at its Drug Substance production facility in Franklin, TN. Following growth and harvest by ABD, ^{13}C-Spirulina Lot API003-006* was shipped as a frozen slurry for lyophilization by Lyophilization Services of New England (LSNE), Manchester, NH.

The egg mix used in the manufacture of the MP meals was prepared by Willamette Egg Farms, Canby, OR, and supplied as pasteurized, formulated liquid egg to OFD, Albany, OR. OFD lyophilized the liquid egg and then bulk packaged ~7.5 kg aliquots into individual polyethylene bags. Each polyethylene bag was tied closed and then inserted into a bulk foil pouch. To prevent spoilage, an oxygen scavenger (approved for food contact) was inserted in each foil pouch prior to sealing.

Bulk ^{13}C-Spirulina Lot API003-006 was then mixed with lyophilized, pasteurized formulated liquid egg by the method of fluid bed granulation at CMIC-VPS, Cranbury, NJ, to produce bulk Drug Product Lot B004-003. Twenty-seven (27) gram aliquots of the dried bulk granulated Drug Product were subsequently unit-dose packaged into foil pouches at Catalent Pharma Solutions, Philadelphia, PA, to yield finished Drug Product Lot D008-002.

Individual sealed pouches of finished Drug Product Lot D008-002 were inserted into MP GEBT test kits during kit assembly at ABD, Brentwood, TN. Twenty-four (24) MP

* As described in Section 9.1.1 of this report, [^{13}C]-*Spirulina platensis* Lot API003-006 was produced with what were, at the time, considered minor adjustments to the [^{13}C]-*Spirulina platensis* manufacturing process. In vitro testing of this lot of drug substance met all Drug Substance specifications. However, results for initial subjects in the present study demonstrated that [^{13}C]-*Spirulina platensis* produced with these adjustments gives a lower ^{13}C in vivo excretion signal. Subsequently a new batch of [^{13}C]-*Spirulina platensis* was prepared using the original manufacturing process and utilized in re-manufacture of MP meals and the re-start of this study. This manufacturing process will be used to manufacture the proposed commercial product.

GEBT kits from kit manufacturing Lot K015-001 were supplied to the study site at Mayo Clinic, Rochester, MN.

9.3.2.2 Identity of Investigational Product Used in Study PRO-CD-005 Re-Start

9.3.2.2.1 Preparation of the Original Process (OP) Meal and GEBT Kits

For the Study PRO-CD-005 Re-Start, the remainder of the 67 OP Meal and GEBT kits utilized in discontinued Study PRO-CD-005 were administered to subjects; see Section 9.3.2.1.1.

9.3.2.2.2 Preparation of Modified Process (MP) Meal and GEBT Kits

Advanced Breath Diagnostics, LLC (ABD) manufactured bulk [^{13}C]-*Spirulina platensis* Lot API003-007, used in the MP meals administered in the Study PRO-CD-005 re-start, according to the intended commercial process at its Drug Substance production facility in Franklin, TN. The same manufacturing process was used to produce the [^{13}C]-*Spirulina platensis* utilized in all OP test meals.

The egg mix used in the manufacture of the MP meals was prepared by Willamette Egg Farms, Canby, OR, and supplied as pasteurized, formulated liquid egg to OFD, Albany, OR. OFD lyophilized the liquid egg and then bulk packaged ~7.5 kg aliquots into individual polyethylene bags. Each polyethylene bag was tied closed and then inserted into a bulk foil pouch. To prevent spoilage, an oxygen scavenger (approved for food contact) was inserted in the each foil pouch prior to sealing.

Following growth and harvest by ABD, ^{13}C-Spirulina Lot API003-007 was shipped as frozen slurry for lyophilization by Lyophilization Services of New England (LSNE), Manchester, NH. Bulk ^{13}C-Spirulina Lot API003-007 was then mixed with lyophilized, pasteurized formulated liquid egg by the method of fluid bed granulation to produce bulk Drug Product Lot B004-005 at CMIC-VPS, Cranbury, NJ. Twenty-seven (27) gram aliquots of the dried bulk granulated Drug Product were subsequently unit-dose packaged into foil pouches at Catalent Pharma Solutions (Philadelphia, PA) to yield finished Drug Product Lot D008-004.

Individual sealed pouches of finished Drug Product Lot D008-004 were inserted into MP GEBT test kits during kit assembly at ABD, Brentwood, TN. Fifty-four (54) MP GEBT kits (49 from Lot K015-002 and 5 from Lot K015-003) containing Drug Product Lot D008-004, were shipped to the study site for use after study re-start.

9.3.3 Selection of Doses

The dose of [^{13}C]-*Spirulina platensis* and the type/amount of food (egg mix, crackers and water) tested in this study were previously determined based upon results obtained in Study PRO-CD-001. See the report for Study PRO-CD-001 for details of how the dose, matrix and caloric value of the GEBT test meal were selected.

OP and MP test meals each contained approximately 100 mg of ^{13}C-labeled *Spirulina platensis* supplying a dose of 43 mg of exogenous ^{13}C in 27 g of egg powder.

9.3.4 Timing of Dose for Each Subject

On each day on which the dual-label or GEBT-only test was to be performed in a given subject, that subject was required to arrive at the clinical site by 8 AM, after an overnight food fast. No solid food or vigorous exercise was permitted during the 8 hours prior to administration of the test meal. No more than 4 fluid ounces of water was permitted between 8 and 1 hours prior to ingestion of the test meal. The test meal was ingested after arrival at the clinical site and collection of baseline breath samples.

9.3.5 Blinding

In this trial, GEBT samples were analyzed and results stored at ABD in Brentwood, TN while scintigraphic data were analyzed and stored at Mayo Clinic in Rochester, MN. To reduce the risk of conscious or unconscious bias in the study, ABD and Mayo personnel were blinded to each other's results until the completion of each Stage of the group-sequential trial. That is, the data were provided to both Mayo and ABD personnel at the time of the Stage 1 interim assessment, after half of the required number of study subjects had been tested (20 by scintigraphy and 44 by GEBT).

9.4 Analytical Performance and Safety Variables

9.4.1 Primary Analytical Performance Variables for GEBT

The primary analytical performance variable for GEBT is the preferred GEBT metric kPCD.

Briefly, the metric kPCD is the Percent Dose (PCD) of ^{13}C excreted in the breath at time t, after consumption of the test meal. To provide a more convenient scale, PCD is multiplied by 1000 to produce kPCD at any time t. The kPCD at any time point and the cumulative kPCD are calculated using the equations and definitions described below.

9.4.1.1 Delta Over Baseline (DOB)

In the GEBT, baseline breath samples are taken before administration of the test meal, and post-meal breath samples are taken at pre-specified times over the course of four hours. The ratio $R = [^{13}CO_2/^{12}CO_2]$ in each breath specimen is measured by GIRMS. The relative difference in this ratio between the post-meal breath specimen (R_{Test}) at any time (t-minutes) and the baseline breath specimen (R_{Base}) is called the Delta Over Baseline (DOB). DOB, in units of delta per mil (symbolized ‰), is the primary measured variable for the GEBT.

$$DOB = \left(\frac{R_{Test} - R_{Base}}{R_S} \right) * 1000 , ‰$$

Where:

R_S = 0.0112372 and is the isotopic ratio ($^{13}CO_2/^{12}CO_2$) in Pee Dee Belemnite (PDB), the reference standard for these measurements.

9.4.1.2 CO_2 Excretion Rate (ER)

The $^{13}CO_2$ Excretion Rate (ER) is the incremental rate (in µmol/min) at which the $^{13}CO_2$ derived from [^{13}C]-*Spirulina platensis* is exhaled at any point in time during a GEBT breath collection process.

$$ER(\mu mol/min) = DOB * R_S * CO_2PR(mmol/min)$$

Where:

CO_2PR (mmol/min), the CO_2 Production Rate, is defined in Section 9.4.1.3.

Also note:

$$ER(mmol/min) = \left(\frac{DOB * Rs * CO_2PR}{1000} \right)$$

$$ER(mg^{13}C/min) = \left(\frac{DOB * Rs * CO_2PR}{1000} \right) * 13$$

9.4.1.3 CO_2 Production Rate (CO_2PR)

The CO_2 Production Rate (CO_2PR), in mmol/min, is calculated from the Basal Metabolic Rate (BMR) estimates based on the Schofield equations which incorporate the subject's gender, age, height and weight.[10]

$$CO_2PR(mmol/min) = BMR * 1.73081$$

Where:

The constant 1.73081 is a factor for converting units,[12]

And

BMR(Megajoules/day) = **c** + [**w** * Weight (in kg)] + [**h*** Height(in meters)]

Where:

The coefficients **c**, **w** and **h** vary with gender and age group (in years) as displayed in Table 9.

Table 9. Coefficients for Use in Determining Basal Metabolic Rate According to the Schofield Equations

	Male			Female		
Age Group (years)	c	w	h	c	w	h
<3	-2.584	0.0007	6.349	-1.730	0.0680	4.281
≥3 to <10	1.736	0.0820	0.545	1.553	0.0710	0.677
≥10 to <18	2.157	0.0680	0.574	0.837	0.0350	1.948
≥18 to <30	2.953	0.0630	-0.042	0.411	0.0570	1.184
≥30 to ≤60	3.670	0.0480	-0.011	3.530	0.0340	0.006
>60	-3.491	0.0380	4.068	0.074	0.0330	1.917

9.4.1.4 Cumulative Mass (mg) of ^{13}C Expired up to Time t (CumER)

CumER (in mg) is the integrated area under the ER (in mg/min) vs time curve at any time point during the GEBT breath collection process. The area under the ER curve is calculated by trapezoidal integration based on the following equation.

$$\text{CumER}_n = \sum_{i=1}^{i=n} \frac{(ER_i + ER_{i-1})(t_i - t_{i-1})}{2}$$

Where:

ER_i and t_i represent the ER_i value (in mg ^{13}C/min) and time (in minutes), respectively, at the i^{th} breath collection time point.

9.4.1.5 Percent Dose of ^{13}C Exhaled Per Minute at Time t (PCD)

The percent dose of ^{13}C exhaled per minute (PCD) at time t is determined according to the following equation:

$$PCD = \frac{DOB * CO_2PR * R_s * 13}{10 * dose}$$

Where:

DOB = measured Delta Over Baseline

CO_2PR = CO_2 Production Rate (in mmol CO_2/min)

R_s = 0.0112372, and is the ratio [$^{13}CO_2/^{12}CO_2$] in the reference standard

13 = the atomic mass of Carbon-13

10 = A constant factor for converting units

dose = the mass (mg) of Carbon-13 in the dose of [^{13}C]-*Spirulina platensis* administered to the test subject in the test meal.

Note: Since PCD values tend to be small numbers (typically less than 0.08), the calculated PCD result is multiplied by 1000 and the result is expressed as kPCD. Thus,

$$kPCD = 1000 * PCD$$

9.4.1.6 Cumulative Percent Dose of ^{13}C Exhaled up to Time t (CumPCD)

The area under the PCD curve is calculated by trapezoidal integration according to the following equation:

$$CUMPCD_n = \sum_{i=1}^{i=n} \frac{(PCD_i + PCD_{i-1})(t_i - t_{i-1})}{2}$$

Where:

PCD_i and t_i represent the PCD value (% dose/min) and time (in minutes), respectively, at the i^{th} breath collection time point.

9.4.2 Primary Analytical Performance Variable for Scintigraphy

For each gamma scintigraphy scan performed at each measured time point, a region of interest (ROI) is drawn around the stomach on the anterior and posterior images for each time frame. Data are corrected for decay of ^{99m}Tc sulphur colloid, the labeling substance used in scintigraphy. To correct for depth or tissue attenuation, the counts of each anterior and posterior ROI are multiplied together, and the square root of the product is taken to obtain the geometric mean. The scintigraphic gastric emptying (GE) metric, $Prop_t$, is the proportion of tracer emptied from the stomach at time t. With a power exponential model, these data are also used to calculate the GE half-time ($t_{1/2}$) after estimating the constants κ and β in the power exponential model, $Prop_t = \exp(-\kappa t^{\beta})$.

9.4.3 Primary Safety Variable

The primary safety assessment was the evaluation of any and all reported adverse events.

9.5 Data Quality Assurance

Advanced Breath Diagnostics, LLC (ABD) developed and provided paper case report forms (CRFs) to the sites for the purpose of collecting study data. Monitoring visits were conducted periodically to review and collect study data. Monitoring visits were conducted per ABD standard operating procedure SOP-CD-001-01, "Monitoring of Clinical Investigations." At each monitoring visit, the study monitor conducted a 100% verification of source data against data in completed CRFs. Whenever possible, errors and omissions were brought to the attention of the responsible study coordinator during monitoring, allowing for immediate correction and verification of accuracy. After the CRF data had been monitored, source verified, and signed by the responsible investigator, photocopies were collected by the study monitor for entry into a secure Excel spreadsheet database maintained by Dr. Martin, the statistician for this study. CRFs were collected for all subjects who signed consent forms. For screen failures, all CRFs applicable to the screening and enrollment visit were completed and subject status as ineligible for inclusion was specified in the disposition portion of the Screening/Enrollment CRF. With the exception of the Final Disposition form, which was completed for all subjects who signed consent forms, other CRFs were marked N/A for "Not Applicable" for screen failures. The monitor conducted source document verification of screen failure reasons.

Query forms were issued to the site prior to any changes in the Excel spreadsheet database due to errors or omissions noted in the CRFs after initial collection by the monitor. Signed and completed query forms were filed with the CRFs at both the site and in the ABD files. For the present study, no query forms were issued.

Dr. Konopka, and an appropriately trained technologist, conducted all breath test analyses at ABD's laboratory. Laboratory results were not transcribed onto a CRF. Rather, laboratory data were entered directly from copies of the original signed laboratory reports into an independent, secure Excel spreadsheet database. Prior to entry into the database, ABD Quality Assurance audited laboratory reports for correctness. After each lab report was generated and reviewed, lab results for each subject were sent electronically to Dr. Martin. Dr. Martin input lab results into a master Excel analytical data spreadsheet, and the compiled data were used for statistical calculations. Data from the two independent spreadsheets were reconciled to insure spreadsheet database accuracy.

9.6 Statistical Methods Planned and Determination of Sample Size

The experimental design and statistical plans are defined in section 5 of the study protocol (see Appendix 15.1, Section 15.1.1); for convenience, they are also provided in the following section of this report.

9.6.1 Statistical Plan

9.6.1.1 Study Design

Study PRO-CD-005 was a single-center, open-label, two-sided test of equivalence of normally distributed continuous variables of unknown variance in a group-sequential, two-treatment, two period crossover trial[11]. In this design, X_i denotes the difference between measured gastric emptying results produced from the modified production process (MP) meal and the original production process (OP) meal [$X_i = (MP)_i - (OP)_I$] for the i^{th} subject receiving the MP meal first. Similarly, Y_i is defined as $Y_i = (MP)_I - (OP)_I$ for the i^{th} subject receiving the OP meal first. After each stage (k = 1 to 2) of the two-stage group-sequential design, the test statistic (θ) was evaluated as the average difference between results from the MP and OP results for the patients tested:

$$\hat{\theta}^{(k)} = \frac{\left(\overline{X}^{(k)} + \overline{Y}^{(k)}\right)}{2}$$

Note: although data for scintigraphic and GEBT measurements were collected simultaneously in dual-label experiments, equivalence determinations of meal effects were performed separately for the two methods.

Following the statistical procedure recommended by Jennison and Turnbull[11], a two-sided equivalence test was constructed satisfying:

$$Pr_{\Theta=\pm\delta}\{\text{declare equivalence}\} \leq \beta$$

for specified δ (i.e., the Margin of Equivalence, defined as the maximum difference in test meal response considered to be inconsequential), and β. The probability, β, represented the "consumer's risk" since wrongly declaring equivalence could lead to inaccurate GEBT results using the MP test meal.

The study was also designed to satisfy the error condition:

$$Pr_{\Theta=0}\{\text{do not declare equivalence}\} \leq \alpha$$

The probability, α, was the "manufacturer's risk" in that declaring a meal difference when in fact there was no difference, would result in an unnecessary and expensive revalidation.

Detailed statistical calculations based on these operational requirements are summarized in the following sections.

9.6.1.2 Margin of Equivalence

Gastric Scintigraphy

Motility Clinic physicians at Mayo advised ABD that, in clinical studies of gastroparetic patients undergoing a variety of therapeutic interventions at Mayo Clinic, a change in scintigraphic $t_{1/2}$ of ± 10% is required to declare a significant change. In Study PRO-CD-003 (GEBT calibration trial) at Mayo Clinic, which utilized the OP test meal, the average $t_{1/2}$ of apparently healthy test subjects was determined to be 66.0 minutes.

Based on this information, $\delta = 6.6$ minutes was adopted as the margin of equivalence for scintigraphic $t_{1/2}$ in the proposed study.

GEBT

A value for the "margin of equivalence" (δ) for the GEBT based on clinical experience is not yet available. To estimate a value, computer simulation calculations were used to study the potential impact of various levels of kPCD bias on % concordance calculated from the data collected in Study PRO-CD-004 (GEBT validation trial). Percent concordance (defined as the overall % diagnostic agreement of the GEBT with scintigraphy) was established as the primary performance measure for the present study. For the simulation calculations, concordance values were based on the trichotomous 95% Biologic Variation cut-off point (COP) limits (for reference, see Appendix 15.2, Section 15.2.9 of the report for Study PRO-CD-004). Results of the simulations for the most informative measurement time points (90 and 120 minutes) are summarized in Table 10.

Table 10. Simulation Results: Effect of kPCD Bias on % Concordance

kPCD Bias (kPCD)	% Concordance	
	90 minute time point	120 minute time point
0.0	100.0	100.0
+ 1.0	98.0	98.1
+2.0	98.0	96.5
+ 3.0	**94.2**	**94.8**
+ 4.0	92.7	93.8
+ 5.0	91.2	92.6
- 1.0	100.0	100.0
- 2.0	100.0	98.0
- 3.0	**100.0**	**95.8**
- 4.0	97.8	92.2
- 5.0	95.3	88.9

Based on these results, a value for the GEBT margin of equivalence (δ) of ± 3.0 kPCD was chosen, as this is the absolute value of the smallest bias at which the point estimate of average concordance, at both the 90 and 120 minute time points, is less than the lower limit of the 95% confidence interval of the zero-bias concordance (i.e., 96.8%).

The margin of equivalence (δ) and σ_D (the estimated 'true' value of the standard deviation of the differences between results of the MP and OP meals calculated from replicate measurements with the OP meal in Studies PRO-CD-002 and PRO-CD-003 of the Investigational Plan) are summarized in Table 11.

Table 11. Margins of Equivalence

Parameter	GEBT (kPCD)	Scintigraphy (fraction emptied)
δ (margin of equivalence)	3.0	6.6
σ_D (StdDev of differences)	9.62	14.3

9.6.2 Statistical Calculations

The trial was planned so that the number of subjects receiving the MP meal first equaled the number of subjects receiving the MP meal second at each stage of the trial. Thus, $n_{Xk} = n_{Yk} = n_k$.

The average difference in results at each stage of the trial was defined as:

$$\hat{\theta}^{(k)} = \frac{\left(\overline{X}^{(k)} + \overline{Y}^{(k)}\right)}{2} \quad \text{with } Var(\hat{\theta}^{(k)}) = \frac{\sigma_D^2}{2n_k}$$

Note that $\overline{X}^{(k)}$ and $\overline{Y}^{(k)}$ represent the means of data accumulated through stage k.

The observed, one-sided t-statistics calculated after each stage of the trial were:

$$\left(T_k^+\right)_{obs} = \frac{\left(\hat{\theta}^{(k)} - \delta\right) * \sqrt{2n_k}}{s_k} \quad \text{and} \quad \left(T_k^-\right)_{obs} = \frac{\left(\hat{\theta}^{(k)} + \delta\right) * \sqrt{2n_k}}{s_k}$$

with $$s_k = \sqrt{\frac{\left(\sum_{i=1}^{n_{Xk}} (X_i - \overline{X}_k)^2 + \sum_{i=1}^{n_{Yk}} (Y_i - \overline{Y}_k)^2\right)}{(n_{Xk} + n_{Yk} - 2)}}$$

With h_k and g_k defined for each k^{th} stage of K stages in terms of the prescribed Δ value and numerically calculated constants $\tilde{C}_{W1}(K, \alpha, \beta, \Delta)$ and $\tilde{C}_{W2}(K, \alpha, \beta, \Delta)$, which ensure Type I and Type II error conditions for the group sequential tests:

$$h_k = -\left(\tilde{C}_{W1} + \tilde{C}_{W2}\right) * (k/K)^{1/2} + \tilde{C}_{W1}(k/K)^{(\Delta - 1/2)} \quad \text{and} \quad g_k = -\tilde{C}_{W2}(k/K)^{(\Delta - 1/2)}$$

Note: Constants $\tilde{C}_{W1}$ and $\tilde{C}_{W2}$ are defined in Table 5.1 of Reference 11.

Two one-sided t-tests were performed after each stage of the trial according to the following rules:

For a two-stage, group sequential trial, after Stage 1,

If $(T_k^+)_{obs} \geq t_{\nu_k,1-\Phi(h_k)}$ **OR** $(T_k^-)_{obs} \leq -t_{\nu_k,1-\Phi(h_k)}$ **STOP, Reject Equivalence**

If $(T_k^+)_{obs} < t_{\nu_k,1-\Phi(g_k)}$ **AND** $(T_k^-)_{obs} > -t_{\nu_k,1-\Phi(g_k)}$ **STOP, Declare Equivalence**

Otherwise, continue to Stage 2.

After Stage 2,

If $(T_K^+)_{obs} \geq t_{\nu_K,1-\Phi(h_K)}$ **OR** $(T_K^-)_{obs} \leq -t_{\nu_K,1-\Phi(h_K)}$ **STOP, Reject Equivalence**

Otherwise, **STOP, Declare Equivalence.**

In these equations, the $t_{\nu_k,(1-\Phi(z))}$ critical values refer to the Student t-deviate corresponding to ν_k degrees of freedom and probability $(1-\Phi(z))$ where $\Phi(z)$ is to the standard normal cumulative distribution function.

9.6.3 Determination of Sample Size

The sample size calculation for the proposed two-stage group sequential trial follows the procedure described in section 6.3.2 of Reference 11. The following parameters were specified to calculate both the scintigraphic and GEBT sample sizes: K (the number of stages) = 2, $\alpha = 0.05$, $\beta = 0.2$ (power = 0.8), and Δ (the Shape parameter of the power family test) = 0.0.

The distribution of test subjects who were planned to undergo scintigraphic testing is summarized in Table 12.

Table 12. Enrollment Plan for Gastric Scintigraphy

Stage	Nx_k *	Ny_k **	Total Subjects	Total Scintigraphic Results
1	10	10	20	40
2 (if necessary)	10	10	20	40
Totals	20	20	40	80

* Nx_k is the number of subjects who were planned to receive the MP meal first.
** Ny_k is the number of subjects who were planned to receive the OP meal first.

The distribution of test subjects who were planned to undergo GEBT testing is summarized in Table 13.

Table 13. Enrollment Plan for the GEBT

Stage	Nx_k *	Ny_k **	Total Subjects	Total GEBT Results
1	22	22	44	88
2 (if necessary)	22	22	44	88
Totals	44	44	88	176

* Nx_k is the number of subjects who were planned to receive the MP meal first.
** Ny_k is the number of subjects who were planned to receive the OP meal first.

9.6.4 Randomization

Normally, randomization of test subjects into one of two groups based on order of test administration in a fixed-size, 2x2 crossover design is a simple process. However, in this study the process was somewhat more complicated in that two test procedures (GEBT and scintigraphy), with different sample size requirements, were simultaneously administered in a group sequential design. Figure 3 depicts the process of randomized testing required to accommodate all possible eventualities that could be encountered to complete the study. Specific randomized test sequences for each of the five possible sequences referenced in Figure 3 are listed in Table 14. In Table 14, **TS #** means Test Subject Number and **Order** indicates the order of test meal administration (Original Process, OP, or Modified Process, MP). Whether the tests administered in the sequence are **Dual-Label** or **GEBT Only** is also indicated in the header.

Figure 3. Randomization of Test Sequences in the Group Sequential Design

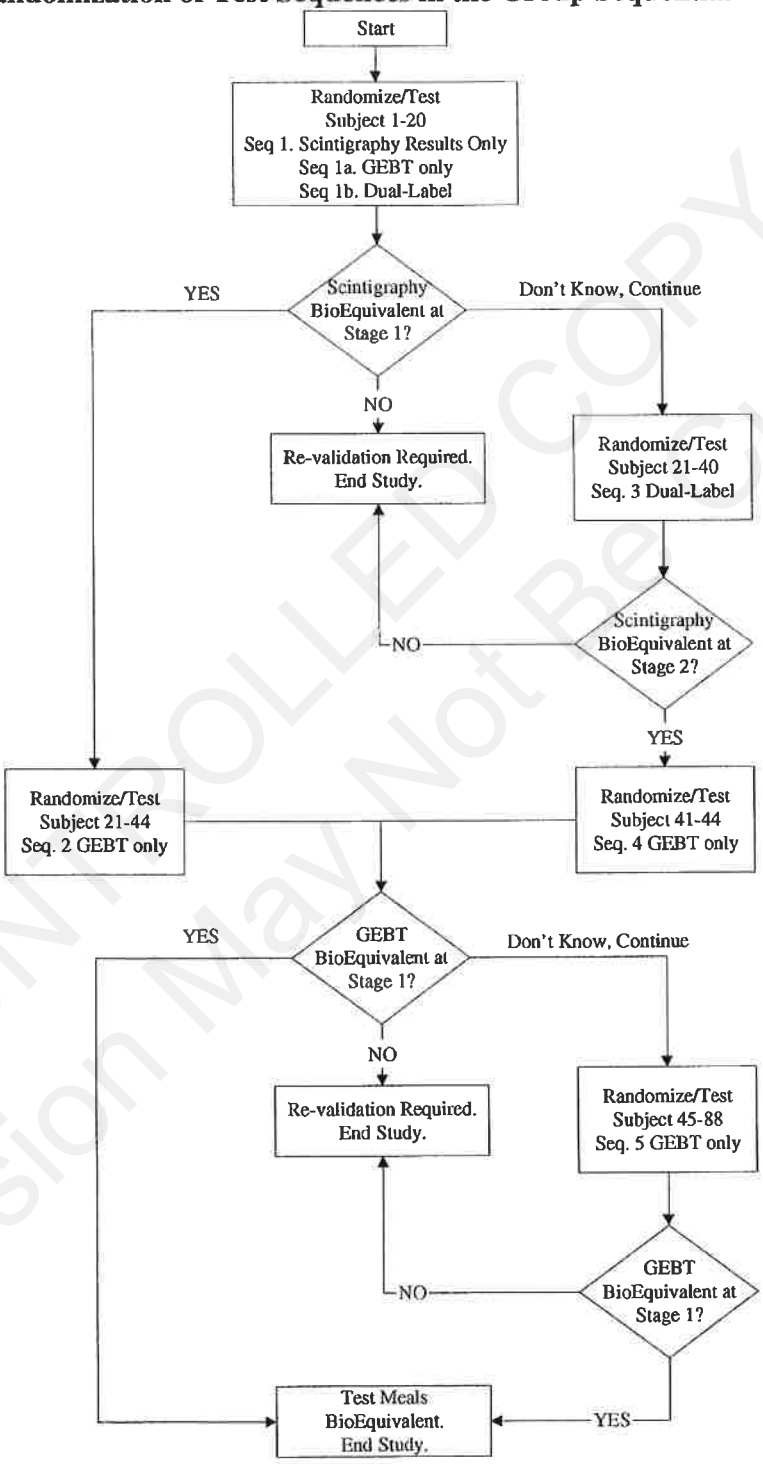

Table 14. Randomized Test Sequences. GEBT Test-Meal Equivalence Study

Sequence #1 (Dual-Label, Only Scintigraphic Data Used)		Sequence #1a (GEBT Only)		Sequence #2 (GEBT Only)		Sequence #3 (Dual-Label)		Sequence #4 (GEBT Only)		Sequence #5 (GEBT Only)	
TS #	Order	TS #	Order	TS #	Order	TS #	Order	TS #	Order	TS #	Order
1	OP/MP	1	OP/MP	21	OP/MP	21	MP/OP	41	MP/OP	45	OP/MP
2	OP/MP	2	OP/MP	22	MP/OP	22	MP/OP	42	OP/MP	46	MP/OP
3	MP/OP	3	MP/OP	23	OP/MP	23	OP/MP	43	OP/MP	47	MP/OP
4	OP/MP	4	OP/MP	24	OP/MP	24	OP/MP	44	MP/OP	48	MP/OP
5	MP/OP	5	MP/OP	25	MP/OP	25	MP/OP			49	OP/MP
6	MP/OP	6	MP/OP	26	MP/OP	26	OP/MP			50	MP/OP
7	OP/MP	7	OP/MP	27	OP/MP	27	MP/OP			51	OP/MP
8	OP/MP	8	OP/MP	28	MP/OP	28	OP/MP			52	OP/MP
9	MP/OP	9	MP/OP	29	OP/MP	29	OP/MP			53	OP/MP
10	MP/OP	10	MP/OP	30	MP/OP	30	MP/OP			54	MP/OP
11	MP/OP	11	MP/OP	31	OP/MP	31	OP/MP			55	OP/MP
12	OP/MP	12	MP/OP	32	OP/MP	32	MP/OP			56	OP/MP
13	OP/MP	13	OP/MP	33	OP/MP	33	OP/MP			57	MP/OP
14	MP/OP	14	MP/OP	34	OP/MP	34	MP/OP			58	OP/MP
		Sequence #1b (Dual-Label)		35	OP/MP	35	MP/OP			59	MP/OP
				36	MP/OP	36	OP/MP			60	MP/OP
		15	MP/OP	37	MP/OP	37	OP/MP			61	OP/MP
		16	OP/MP	38	OP/MP	38	MP/OP			62	OP/MP
		17	OP/MP	39	MP/OP	39	OP/MP			63	OP/MP
		18	OP/MP	40	OP/MP	40	MP/OP			64	OP/MP
		19	MP/OP	41	MP/OP					65	MP/OP
		20	OP/MP	42	MP/OP					66	MP/OP
				43	MP/OP					67	OP/MP
				44	MP/OP					68	MP/OP
										69	MP/OP
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										85	OP/MP
										86	OP/MP
										87	MP/OP
										88	MP/OP

9.7 Changes of Planned Conduct of the Study

The original version of the protocol for this study was PRO-CD-005-01. This version of the protocol was reviewed and approved by the Mayo Clinic IRB responsible for oversight. The present study was initiated under this protocol. As described previously in section 9.1.1 of this report, initial study activities were discontinued after 14 subjects were tested by the dual-label (GEBT and scintigraphy) method. Scintigraphic testing of these 14 subjects was valid but GEBT results were non-evaluable.

Test Meal Equivalence Study PRO-CD-005 was re-started under revised protocol PRO-CD-005-02. The amended protocol differed from the original only in the testing prescribed for Sequence 1 of the randomized test sequences, which are displayed in Table 3 of this report and in Section E of the study protocol (Appendix 15.1, Section 15.1.1). In original protocol PRO-CD-005-01, Sequence 1 consisted of testing each of 20 Normal subjects with a dual-label test meal to acquire both scintigraphic and GEBT results for each test meal administration. It was concluded that scintigraphic results collected for the first 14 test subjects who received dual-label test meals during the discontinued portion of Study PRO-CD-005 represent valid scintigraphic comparisons between the OP and MP test meals, making it unnecessary to repeat dual-label testing on the first 14 subjects in the Study PRO-CD-005 re-start. In revised Sequence 1a, GEBT-only tests (no dual-label tests) were performed for the first 14 test subjects. To fulfill the study requirement for dual-label testing of 20 subjects, subjects 15-20 were planned to undergo dual-label testing under revised Sequence 1a of the Study PRO-CD-005 Re-start. Protocol PRO-CD-005-02 insured continued proper randomization of test subjects and OP/MP test administration sequences. The amended protocol was reviewed and approved by the Mayo Clinic IRB responsible for oversight.

One final amendment was made to the protocol to revise wording to more clearly indicate that a urine pregnancy test was required within 48 hours prior to each Gastric Emptying test administration, whether test administration was by the dual-label procedure, or by GEBT only. The revised language is specified in study protocol PRO-CD-005-03, which was reviewed and approved by the Mayo Clinic IRB responsible for oversight.

SECTION 10 STUDY TEST SUBJECTS

10.1 Disposition of Test Subjects

10.1.1 Initial Discontinued Study PRO-CD-005

The study was initiated under Protocol PRO-CD-005-01. As described in Section 9.1.1 of this report, the protocol was discontinued early after 14 subjects completed both the MP and OP dual-label test procedures. Scintigraphic test results on the 14 patients were valid, but GEBT test results were not.

In the initial portion of this study, 18 subjects were screened. One subject, 05-MC-012, began the screening process but declined to provide consent, and was not enrolled. Two (2) subjects, 05-MC-013 and 05-MC-016, did not meet screening criteria. One (1) subject, 05-MC-005, withdrew consent prior to undergoing the dual-label testing procedure. The remaining 14 subjects were enrolled and completed dual-label testing per protocol PRO-CD-005-01.

10.1.2 Study PRO-CD-005 Re-start

The study was re-started under protocol version PRO-CD-005-02. As fully described in the study design section of this report (Section 9.6.1), the trial was a group-sequential, two-treatment, two-period crossover trial. Interim assessment was allowed to determine whether equivalence criteria were met at a Stage 1 stopping point. The study called for a total of 88 test subjects, 40 of whom were required to undergo dual-label testing to assess scintigraphic equivalence; GEBT assessment was required for all 88 subjects. Interim assessment at Stage 1 was allowed after half of the required number of study subjects had been tested (20 by scintigraphy and 44 by GEBT).

To meet the Stage 1 scintigraphic testing requirement of 20 participants, 6 new subjects, in addition to the 14 subjects tested under protocol version PRO-CD-005-01, were tested by the dual-label procedure (once with an MP meal and on a separate occasion with an OP meal). To meet the requirement of 44 GEBT subjects, GEBT results (MP and OP) were acquired concurrently from the 6 subjects administered dual-label tests and an additional 38 subjects who received MP and OP meals and underwent GEBT testing only.

Overall, 55 subjects were screened in the Study PRO-CD-005 Re-start. Three subjects, 05-MC-044, 05-MC-055, and 05-MC-61Rp1*, completed the screening process and were considered eligible based on screening results but withdrew consent before they were enrolled. Of the 52 subjects were enrolled, 4 of whom withdrew or were dismissed for the following reasons.

*Rp means test subject was replacement for original subject. Rp1 is the first replacement subject, Rp2 the second replacement, etc.

- Subject 05-MC-019 received the test meal once at Visit 2, but test results from that administration were non-evaluable due to two protocol deviations (see Section 10.2). Subject 05-MC-019 was given the option to re-enroll and repeat all testing, but declined to do so, and was dismissed from the study prior to Visit 3 (second test meal administration).
- Two subjects, 05-MC-061 and 05-MC-061-Rp2, withdrew after Visit 2 due to adverse events unrelated to the study device; each received the GEBT test meal on one occasion at Visit 2.
- One subject, 05-MC-044, was dismissed from the study when the study coordinator was notified that he/she was participating simultaneously in another study.

Forty-eight (48) subjects completed testing as specified in the study protocol. Of those 48 subjects, results for 4 subjects were considered non-evaluable as follows.

- Baseline breath samples were incorrectly collected at both test administration visits for Subject 05-MC-020 (see Section 10.2). This issue was promptly reported by the study coordinator and Subject 05-MC-020 was given the option to re-enroll and repeat all testing, but declined to do so.
- GEBT results for subjects 05-MC-027, 05-MC-028, and 05-MC-029 were deemed non-evaluable due to breath collection timers being mistakenly set prior to meal consumption (instead of being set immediately after the meal was consumed, per the GEBT instructions). This issue was promptly reported by the study coordinator and these subjects were re-screened, re-enrolled under subject IDs 05-MC-027-R1[†], 05-MC-028-R1, and 05-MC-029-R1 and subsequently re-administered both MP and OP GEBT test meals per the study protocol.

Thus, of 48 subjects who completed all testing as required by the study protocol, results for 44 of those subjects were considered evaluable.

Tables 15.2-2 and 15.2-11 in Listing 15.2.4 of Appendix 15.2 provide a complete listing of the disposition of all subjects screened and enrolled in Study PRO-CD-005.

[†] R means repeat of testing and analysis of a test subject. R1 is the first repeat, R2 is the second repeat, etc.

10.2 Protocol Deviations

There were nine protocol deviations involving six subjects (see Appendix 15.2, Listing 15.2.2). For convenience, they may be summarized as follows:

- For subjects 05-MC-019 and 05-MC-020, the GEBT baseline breath sample was incorrectly collected after meal consumption (the correct procedure is to collect the baseline breath sample prior to test meal consumption).
- Subject 05-MC-019 was administered the first GEBT test meal (not dual-labeled) prior to confirmation of a negative pregnancy test.
- For subjects 05-MC-027, 05-MC-028 and 05-MC-029, the breath collection timer was incorrectly set prior to test meal consumption at Visit 2 (the correct procedure is to set the timer immediately after the meal is consumed).
- Following incorrect breath sample collection at Visit 2, subjects 05-MC-028 and 05-MC-029 completed Visit 3 (with correct test administration), but to include Visit 3 results without repeating both visits would have violated test sequence requirements.
- Subject 05-MC-044 reported receiving NO investigational drugs within 4 weeks of the study participation. However, it was discovered that this subject had participated in an endocrine study within the past 30 days in which the subject had received a Lupron injection and an estrogen patch.

10.3 Test Subject Complaints

No test subject complaints were reported.

SECTION 11 ANALYTICAL PERFORMANCE EVALUATION

11.1 Data Sets Analyzed

Discontinued Study PRO-CD-005

Fourteen (14) subjects completed dual-label testing requirements in the discontinued portion of study PRO-CD-005. Scintigraphic data for all 14 subjects were valid and used for analysis. However, due to early findings that GEBT results obtained with the modified process (MP) test meals were significantly lower than results from the original process (OP) test meals, all GEBT data from these first 14 subjects were rejected (see Section 9.1.1 of this report).

Scintigraphic data from both dual-label test meal administrations to each of the 14 subjects were combined with scintigraphic data from 6 additional dual-label test subjects enrolled in the Study PRO-CD-005 Re-start to assess scintigraphic equivalence of OP and MP test meals.

Study PRO-CD-005 Re-start

Forty-five (45) subjects completed the Study PRO-CD-005 Re-start and data from 44 of those subjects were available for analysis. Data from one subject, 05-MC-020, were rejected due to an error in baseline breath sample collection at both test meal administrations (see Section 10.2).

Data from both test meal administrations to each of the 44 subjects were used to assess the equivalence of test meals manufactured by a modified process (MP) and test meals manufactured by the original process (OP). Scintigraphic data from both test meal administrations for 6 subjects who received dual-labeled test meals were combined with like data from the discontinued portion of Study PRO-CD-005 to assess scintigraphic equivalence of OP and MP test meals.

11.2 Demographic Characteristics

The following data and information collected at screening (Visit 1) for all subjects enrolled in initial study PRO-CD-005 and then the study PRO-CD-005 Re-start are provided in Appendix 15.2, Listing 15.2.4.

- Demographic information for subjects screened for enrollment in this study
- Results for Inclusion/Exclusion Criteria assessments for subjects screened for enrollment in this study
- Medical history results for subjects enrolled in this study
- Results of physical examinations performed on subjects enrolled in this study
- Concomitant medications recorded at screening or during the study

Additionally, vital signs and pregnancy test results collected prior to test meal administration at Visits 2 and 3 are provided in Appendix 15.2, Listing 15.2.4.

11.3 Measurements of GEBT Test Compliance

Discontinued Study PRO-CD-005

All 14 subjects who enrolled in the discontinued portion of this study and, based on screening results, were eligible to participate, proceeded to receive all assigned test meals and provided breath samples at all designated times. In addition, one subject did not provide consent, one withdrew consent prior to undergoing any testing, and two subjects failed screening; these four subjects are not included in the 14 subjects who participated in this portion of the study.

Study PRO-CD-005 Re-start

Forty-five (45) of the 52 subjects who enrolled in the Study PRO-CD-005 Re-start and, based on screening results, were eligible to participate, proceeded to receive all assigned test meals and provided breath samples at all designated times. One subject, 05-MC-019, received the test meal once at Visit 2, but test results from that administration were non-evaluable due to two protocol deviations (see Section 10.2). Subject 05-MC-019 was given the option to re-enroll and repeat all testing, but declined to do so, and was dismissed from the study prior to Visit 3 (second test meal administration). Two (2) subjects, 04-MC-044-Rp1 and 05-MC-055 withdrew after enrollment but prior to any test meal administrations. Two (2) subjects, 05-MC-061 and 05-MC-061-Rp2, withdrew after Visit 2 due to adverse events unrelated to the study device; each received the GEBT test meal on one occasion at Visit 2. One (1) subject, 05-MC-061-Rp1, completed screening but withdrew consent and was not enrolled. One (1) subject, 05-MC-044, received the test meal on one occasion (Visit 2), but was dismissed from the study when the study coordinator was notified that he/she was participating simultaneously in another study.

11.4 Analytical Performance Results and Tabulations of Test Subject Data

11.4.1 Analysis of Analytical Performance

11.4.1.1 Test Meal Equivalence as Assessed by Scintigraphy

The $t_{1/2}$ values obtained on each test subject were calculated using the power-exponential curve fitting model (Table Curve 2D, v.5.01). Scintigraphic test results from each subject for each test meal were tabulated after Stage 1 was completed, i.e., scintigraphic test data were available for 20 subjects and GEBT data were available for 44 subjects. The entire data set is found in Appendix 15.2, Listing 15.2.8 of this report. Test results from each subject for each test meal are shown in Table 15. Note that the average $t_{1/2}$ values for MP versus OP are very similar with like inter-subject standard deviations. The average $t_{1/2}$ Δ

in MP vs. OP was 2.8 minutes, less than half of the specified margin of equivalence defined for scintigraphy (6.6 minutes).

Table 15. Scintigraphy Data: Test Meal Equivalence Study PRO-CD-005, N=20

Count	Meal Sequence	Subject ID	Stage	$t_{1/2}$ MP (minutes)	$t_{1/2}$ OP (minutes)	(MP-OP) (minutes)
1	MP/OP	05-MC-001	1	47.4	52.2	-4.8
2	MP/OP	05-MC-002	1	48.8	66.1	-17.3
3	OP/MP	05-MC-004	1	57.4	67.2	-9.8
4	MP/OP	05-MC-007	1	56.9	61.7	-4.8
5	OP/MP	05-MC-006	1	83.3	72.1	11.2
6	OP/MP	05-MC-010	1	69.4	70.2	-0.8
7	MP/OP	05-MC-011	1	63.1	52.3	10.8
8	MP/OP	05-MC-008	1	60.6	70.7	-10.1
9	OP/MP	05-MC-009	1	54.5	61.5	-7.0
10	OP/MP	05-MC-015	1	95.5	88.2	7.3
11	OP/MP	05-MC-017	1	76.3	95.9	-19.6
12	OP/MP	05-MC-003	1	79.6	76.5	3.1
13	MP/OP	05-MC-018	1	58.8	72.6	-13.8
14	OP/MP	05-MC-014	1	57.9	39.2	18.7
15	OP/MP	05-MC-033	1	84.9	81.6	3.3
16	MP/OP	05-MC-036	1	43.2	41.5	1.7
17	MP/OP	05-MC-037	1	47.2	56.0	-8.8
18	MP/OP	05-MC-038	1	69.0	77.4	-8.4
19	OP/MP	05-MC-039	1	55.8	50.0	5.8
20	MP/OP	05-MC-040	1	52.8	64.6	-11.8
			Average	63.1	65.9	-2.8
			SD	14.3	14.7	10.3

Using these data, calculations necessary to execute the two-sided test of equivalence required to declare meal equivalence were performed as described in Protocol PRO-CD-005-03, Sections 5(A) and 5(F) (see Appendix 15.1, Section 15.1.1), and in Section 9.6.1 of this report. For clarity, key elements of the statistical design and calculations are re-stated in the following paragraphs, followed by a summary of the results of statistical calculations. The calculations clearly indicate that, after Stage 1, the statistical criteria required to declare meal equivalence by the scintigraphic method were met and no further testing by scintigraphy was required.

In the statistical calculations and equations, X_i denotes the difference between measured gastric emptying results obtained from the Modified Production (MP) meal and the Original Production (OP) meal [$X_i = (MP)_i - (OP)_I$] for the i^{th} subject receiving the MP meal first. Similarly, Y_i is defined as $Y_i = (MP)_I - (OP)_I$ for the i^{th} subject receiving the OP meal first.

After each stage (k = 1 to 2) of the two-stage group-sequential design, the test statistic (θ) was evaluated as the average difference between the MP and OP results for the patients tested:

$$\hat{\theta}^{(k)} = \frac{\left(\overline{X}^{(k)} + \overline{Y}^{(k)}\right)}{2}$$

Note: although data for scintigraphic and GEBT measurements were collected simultaneously in dual-label test procedures, equivalence determinations of meal effects were performed separately for the two methods.

Following the statistical procedure recommended by Jennison and Turnbull[11], a two-sided equivalence test was constructed satisfying:

$$\Pr_{\Theta=\pm\delta}\{\text{declare equivalence}\} \leq \beta$$

for specified δ (i.e., the Margin of Equivalence, defined as the maximum difference in test meal response considered to be inconsequential*) and β. The probability, β, represents the "consumer's risk" since wrongly declaring equivalence could lead to inaccurate GEBT results using the MP test meal.

The following error condition was also required to be satisfied:

$$\Pr_{\Theta=0}\{\text{do not declare equivalence}\} \leq \alpha$$

The probability, α, is the "manufacturer's risk" in that declaring a meal difference when in fact there is no difference, would result in an unnecessary and expensive revalidation.

Detailed statistical calculations based on these operational requirements, and the appropriate times at which test meal equivalence may be accepted or must be rejected, are described below. Equations in this section can be simplified by noting that the trial was planned such that the number of subjects receiving the MP meal first equals the number of subjects receiving the MP meal second at each stage of the trial. Thus, $n_{Xk} = n_{Yk} = n_k$.

* In regards to adopting a margin of equivalence for scintigraphy ($t_{1/2}$) for these calculations, Motility Clinic physicians at Mayo advised ABD that, in clinical studies of gastroparetic patients undergoing a variety of therapeutic interventions at Mayo Clinic, a change in scintigraphic $t_{1/2}$ of ± 10% is required to declare a significant change. In Study PRO-CD-003, completed at Mayo Clinic using the Original Process (OP) test meal, the average $t_{1/2}$ of healthy test subjects was determined to be 66.0 minutes. A listing of the $t_{1/2}$ values for subjects undergoing dual label testing in Study PRO-CD-003 may be found in Appendix 15.2, Listing 15.2.9 of this report. Based on this information, ABD adopted δ = 6.6 minutes as the margin of equivalence for scintigraphic $t_{1/2}$ in this study. As previously shown in Table 10, the average intra-subject $t_{1/2}$ Δ was only 2.8 minutes vs. a margin of equivalence limit of 6.6 minutes in Stage 1 of this study.

The average difference in results at each stage of the trial was defined as:

$$\hat{\theta}^{(k)} = \frac{\left(\overline{X}^{(k)} + \overline{Y}^{(k)}\right)}{2} \quad \text{with} \quad Var(\hat{\theta}^{(k)}) = \frac{\sigma_D^2}{2n_k}$$

Note that $\overline{X}^{(k)}$ and $\overline{Y}^{(k)}$ represent the means of data accumulated through stage k.

The observed, one-sided t-statistics calculated after each stage of the trial were:

$$\left(T_k^+\right)_{obs} = \frac{\left(\hat{\theta}^{(k)} - \delta\right) * \sqrt{2n_k}}{s_k} \quad \text{and} \quad \left(T_k^-\right)_{obs} = \frac{\left(\hat{\theta}^{(k)} + \delta\right) * \sqrt{2n_k}}{s_k}$$

$$\text{with} \quad s_k = \sqrt{\frac{\left(\sum_{i=1}^{n_{Xk}} (X_i - \overline{X}_k)^2 + \sum_{i=1}^{n_{Yk}} (Y_i - \overline{Y}_k)^2\right)}{\left(n_{Xk} + n_{Yk} - 2\right)}}$$

With h_k and g_k defined as follows for each k^{th} stage of K stages in terms of the prescribed Δ value and numerically calculated constants $\tilde{C}_{W1}(K,\alpha,\beta,\Delta)$ and $\tilde{C}_{W2}(K,\alpha,\beta,\Delta)$, which ensure Type I and Type II error conditions for the group sequential tests:

$$h_k = -\left(\tilde{C}_{W1} + \tilde{C}_{W2}\right) * (k/K)^{1/2} + \tilde{C}_{W1}(k/K)^{(\Delta - 1/2)} \quad \text{and} \quad g_k = -\tilde{C}_{W2}(k/K)^{(\Delta - 1/2)}$$

Note: Constants $\tilde{C}_{W1}$ and $\tilde{C}_{W2}$ are listed in Table 5.1, Reference 11.

Two one-sided t-tests were performed after each stage of the trial, according to the following rules:

For a two-stage, group sequential trial, after stage 1,

If $\left(T_k^+\right)_{obs} \geq t_{\nu_k, 1-\Phi(h_k)}$ **OR** $\left(T_k^-\right)_{obs} \leq -t_{\nu_k, 1-\Phi(h_k)}$ **STOP, Reject Equivalence**

If $\left(T_k^+\right)_{obs} < t_{\nu_k, 1-\Phi(g_k)}$ **AND** $\left(T_k^-\right)_{obs} > -t_{\nu_k, 1-\Phi(g_k)}$ **STOP, Declare Equivalence**

Otherwise, continue to stage 2.

After stage 2,

If $\left(T_K^+\right)_{obs} \geq t_{\nu_K, 1-\Phi(h_K)}$ **OR** $\left(T_K^-\right)_{obs} \leq -t_{\nu_K, 1-\Phi(h_K)}$ **STOP, Reject Equivalence**

Otherwise, **STOP, Declare Equivalence.**

Scintigraphic Equivalence Calculation Results:

The X and Y grouped data required for performing Stage 1 calculations cited above are tabulated in Tables 16 and 17, respectively. All subjects whose data are included in the X group data set received the MP meal at Visit 2 and the OP meal at Visit 3. All subjects whose date are included in the Y group set received the OP meal at Visit 2 and the MP meal at Visit 3.

Table 16. Scintigraphic X Group Data for Test Meal Equivalence Calculations (data expressed as half emptying time, or $t_{1/2}$, in minutes)

Experimental Data X Group Data. Sequence A:B					
Subject #	**Stage**	**A(test) (minutes)**	**B(ref) (minutes)**	**X=A-B (minutes)**	**Count**
05-MC-004	1	57.4	67.2	-9.8	1
05-MC-006	1	83.3	72.1	11.2	2
05-MC-010	1	69.4	70.2	-0.8	3
05-MC-009	1	54.5	61.5	-7.0	4
05-MC-015	1	95.5	88.2	7.3	5
05-MC-017	1	76.3	95.9	-19.6	6
05-MC-003	1	79.6	76.5	3.1	7
05-MC-014	1	57.9	39.2	18.7	8
05-MC-033	1	84.9	81.6	3.3	9
05-MC-039	1	55.8	50.0	5.8	10

Table 17. Scintigraphic Y Group Data for Test Meal Equivalence Calculations (data expressed as half emptying time, or $t_{1/2}$, in minutes)

Experimental Data Y Group Data. Sequence B:A					
Subject #	**Stage**	**A(test) (minutes)**	**B(ref) (minutes)**	**Y=A-B (minutes)**	**Count**
05-MC-001	1	47.4	52.2	-4.8	1
05-MC-002	1	48.8	66.1	-17.3	2
05-MC-007	1	56.9	61.7	-4.8	3
05-MC-011	1	63.1	52.3	10.8	4
05-MC-008	1	60.6	70.7	-10.1	5
05-MC-018	1	58.8	72.6	-13.8	6
05-MC-036	1	43.2	41.5	1.7	7
05-MC-037	1	47.2	56.0	-8.8	8
05-MC-038	1	69.0	77.4	-8.4	9
05-MC-040	1	52.8	64.6	-11.8	10

Using a δ (margin of equivalence limit) of 6.6 minutes, the calculated experimental design limits for accepting or rejecting scintigraphic test meal equivalence after Stage 1 were as follows:

Experimental Design				
δ = 6.6			Acceptance Limit	Rejection Limit
Stage	Nx_k	Ny_k	T^+_k	T^+_k
1	10	10	-1.395	0.713

Using the experimental data from Stage 1, the calculated values met the criteria for equivalence as shown below.

Results (Observed)			
Stage	T^+_{obs}	T^-_{obs}	Action
1	-4.319	1.775	**Accept Equivalence**

That is, the study data and calculations satisfied the statistical criteria that:

If the observed T^+_k < the Stage 1 acceptance limit of -1.395
AND
If the observed T^-_k > - (the Stage 1 acceptance limit of 0.713)
THEN ACCEPT EQUIVALENCE.

Conclusion: GEBT test meals prepared by the Modified Process (MP) and the Original Process (OP) empty from the human stomach of adults at equivalent rates as measured by gastric scintigraphy.

11.4.1.2 Test Meal Equivalence as Assessed by GEBT

The GEBT (kPCD) values were tabulated at each measured time point (45, 90, 120, 150, and 180 minutes) after Stage 1 for all 44 GEBT participants. The entire data set is found in Appendix 15.2, Listing 15.2.8 of this report.

The mean kPCD values for the respective OP and MP meals administered to the 44 GEBT test subjects in Stage 1 are shown in Table 18. Although the 90 and 120 minute measurement times are the critical times for calculating equivalence statistics, the average GEBT (kPCD) values for MP and OP are very close at all measurement times.

Table 18. Mean GEBT (kPCD) Values for OP and MP Test Meals: N=44

Measurement Time	45 Minutes	90 Minutes	120 Minutes	150 Minutes	180 Minutes
Mean: MP Meal	19.57	43.51	55.21	59.17	58.92
Mean: OP Meal	21.25	44.10	55.28	59.19	58.38
Average Δ (MP-OP)	-1.68	-0.59	-0.07	-0.02	0.54

The average intra-patient difference (Δ) in MP vs. OP meals was only 0.59 and 0.07 kPCD at the critical 90 and 120 minute measurement times, respectively. This is

significantly less than the specified δ (margin of equivalence defined for GEBT) of ± 3.0 kPCD.

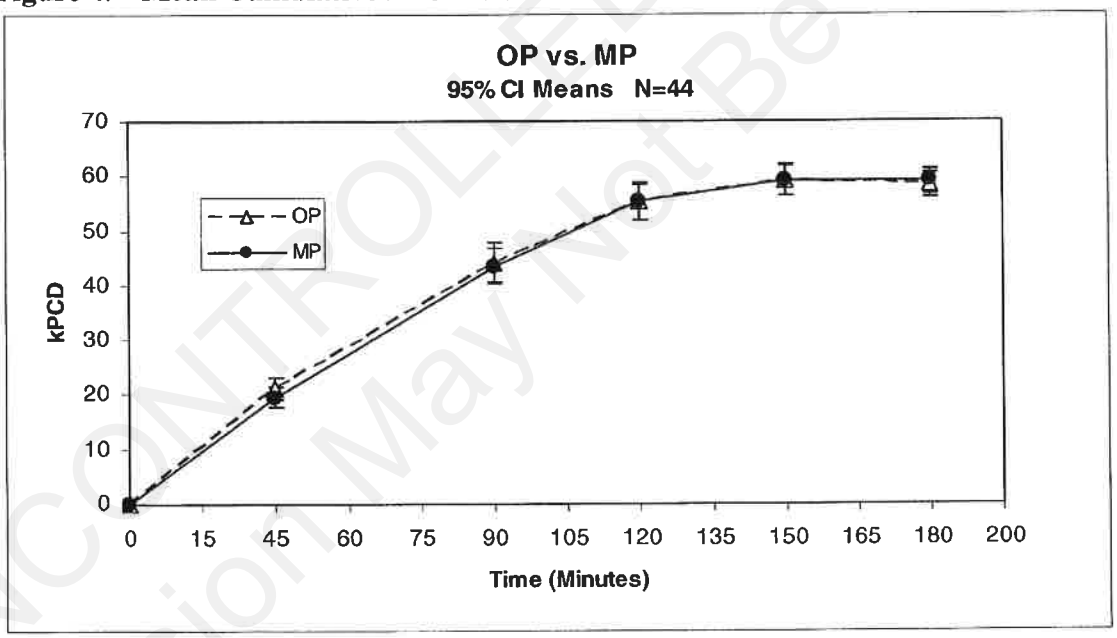
Also, the inter-subject standard deviation observed for MP and OP meals was very close at each measurement time, as presented in Table 19.

Table 19. Standard Deviations (SD) Observed for Respective MP and OP Meals: N=44

Measurement Time	45 Minutes	90 Minutes	120 Minutes	150 Minutes	180 Minutes
SD: MP Meal	6.38	11.10	11.50	9.56	7.69
SD: OP Meal	7.11	11.83	11.05	9.12	7.54
SD: Δ (MP-OP)	6.39	8.54	8.48	7.42	6.75

Figure 4 demonstrates the virtual equivalence of the two meals.

Figure 4. Mean Cumulative kPCD Values for OP vs. MP Test Meals



Utilizing the GEBT (kPCD) data from the 44 subjects at the 90 and 120 minute measurement times, calculations necessary to execute the two-sided test of equivalence required to declare meal equivalence were performed as described in PRO-CD-005-03, Sections 5(A) and 5(F) (see Appendix 15.1, Section 15.1.1), and in Section 9.6.1 of this report. The exact same calculations as those conducted for assessing scintigraphic meal equivalence in Section 11.4.1.1 were conducted for the GEBT at both the 90 and 120 minute time points. As with the scintigraphic method, the calculations clearly indicated that, after Stage 1, the statistical criteria required to declare meal equivalence by the GEBT method were met and no further testing of the two GEBT test meals was required.

90 Minute GEBT Measurement Time Point:

The X and Y grouped data required for performing Stage 1 GEBT equivalence calculations at the 90 minute time point are tabulated in Tables 20 and 21, respectively. All subjects whose data is included in the X group data set received the MP meal at Visit 2 and the OP meal at Visit 3. All subjects whose data is included in the Y group set received the OP meal at Visit 2 and the MP meal at Visit 3.

Table 20. 90 Minute GEBT X Group Data for Test Meal Equivalence Calculations

Experimental Data X Group Data. Sequence A:B					
Subject #	**Stage**	**A(test) (kPCD)**	**B(ref) (kPCD)**	**X=A-B (kPCD)**	**Count**
05-MC-023	1	60.70	71.97	-11.27	1
05-MC-026	1	54.76	48.10	6.66	2
05-MC-025	1	60.18	46.51	13.67	3
05-MC-029-R1	1	53.46	62.20	-8.74	4
05-MC-030	1	50.24	42.73	7.51	5
05-MC-031	1	34.87	37.32	-2.45	6
05-MC-032	1	40.74	44.23	-3.49	7
05-MC-035	1	48.06	61.22	-13.16	8
05-MC-033	1	34.03	28.57	5.46	9
05-MC-039	1	51.55	57.82	-6.27	10
05-MC-042	1	53.05	51.33	1.72	11
05-MC-046	1	43.59	52.36	-8.77	12
05-MC-047	1	29.09	37.95	-8.86	13
05-MC-049	1	37.82	32.79	5.03	14
05-MC-051	1	30.46	27.46	3.00	15
05-MC-057	1	50.01	38.23	11.78	16
05-MC-061-Rp2	1	42.02	33.53	8.49	17
05-MC-052	1	32.10	50.54	-18.44	18
05-MC-043	1	36.18	35.35	0.83	19
05-MC-058	1	22.21	27.00	-4.79	20
05-MC-065	1	24.02	32.58	-8.56	21
05-MC-063	1	27.83	25.40	2.43	22

Table 21. 90 Minute GEBT Y Group Data for Test Meal Equivalence Calculations

Experimental Data Y Group Data. Sequence B:A					
Subject #	**Stage**	**A(test) (kPCD)**	**B(ref) (kPCD)**	**Y=A-B (kPCD)**	**Count**
05-MC-021	1	40.07	37.35	2.72	1
05-MC-022	1	52.62	48.87	3.75	2
05-MC-024	1	38.14	32.02	6.12	3
05-MC-027-R1	1	26.73	35.60	-8.87	4
05-MC-028-R1	1	39.03	36.12	2.91	5
05-MC-034	1	45.43	46.84	-1.41	6
05-MC-036	1	57.43	63.20	-5.77	7
05-MC-037	1	52.75	59.35	-6.60	8
05-MC-038	1	54.21	44.03	10.18	9
05-MC-040	1	64.47	66.99	-2.52	10
05-MC-041	1	39.81	44.01	-4.20	11
05-MC-044-Rpl	1	44.53	57.92	-13.39	12
05-MC-045	1	54.09	48.31	5.78	13
05-MC-048	1	41.71	35.25	6.46	14
05-MC-050	1	37.42	31.95	5.47	15
05-MC-053	1	36.51	35.81	0.70	16
05-MC-054	1	56.24	38.13	18.11	17
05-MC-056	1	41.01	37.56	3.45	18
05-MC-059	1	49.92	64.08	-14.16	19
05-MC-060	1	56.96	46.90	10.06	20
05-MC-062-R1	1	21.06	37.62	-16.56	21
05-MC-064	1	47.12	47.06	0.06	22

Using a δ (margin of equivalence limit) of ± 3.0 kPCD, the calculated experimental design limits for accepting or rejecting GEBT test meal equivalence at the 90 minute measurement time, after Stage 1, are as follows:

Experimental Design				
δ = 3.0			Acceptance Limit	Rejection Limit
Stage	**Nx_k**	**Ny_k**	**T^+_k**	**T^+_k**
1	22	22	-1.363	0.704

Using the experimental data from Stage 1, the calculated values met the criteria for GEBT equivalence at 90 minutes as shown below.

Results (Observed)			
Stage	**T^+_{obs}**	**T^-_{obs}**	**Action**
1	-2.765	1.857	**Accept Equivalence**

That is, the GEBT study data and calculations for the 90 minute measurement time point satisfied the following statistical criteria.

If the observed T^{+}_{k} < the Stage 1 acceptance limit of -1.363
AND
If the observed T^{-}_{k} > - (the Stage 1 acceptance limit of 0.704)
THEN ACCEPT EQUIVALENCE.

120 Minute Measurement Time Point:

The equivalence calculations were likewise conducted for the 120 minute time point. The X and Y grouped GEBT data for the 120 minute measurement time are tabulated in Tables 22 and 23, respectively.

Table 22. 120 Minute GEBT X Group Data for Test Meal Equivalence Calculations

Experimental Data X Group Data. Sequence A:B					
Subject #	**Stage**	**A(test) (kPCD)**	**B(ref) (kPCD)**	**X=A-B (kPCD)**	**Count**
05-MC-023	1	72.10	73.16	-1.06	1
05-MC-026	1	72.83	62.33	10.50	2
05-MC-025	1	64.72	53.01	11.71	3
05-MC-029-R1	1	70.06	72.94	-2.88	4
05-MC-030	1	63.14	61.02	2.12	5
05-MC-031	1	52.15	51.32	0.83	6
05-MC-032	1	57.15	61.81	-4.66	7
05-MC-035	1	59.12	70.68	-11.56	8
05-MC-033	1	42.41	40.59	1.82	9
05-MC-039	1	58.41	59.81	-1.40	10
05-MC-042	1	59.18	62.63	-3.45	11
05-MC-046	1	55.61	68.75	-13.14	12
05-MC-047	1	42.30	49.12	-6.82	13
05-MC-049	1	52.70	48.52	4.18	14
05-MC-051	1	44.10	38.29	5.81	15
05-MC-057	1	61.97	48.34	13.63	16
05-MC-061-Rp2	1	53.40	46.40	7.00	17
05-MC-052	1	37.06	61.26	-24.20	18
05-MC-043	1	48.40	43.04	5.36	19
05-MC-058	1	34.10	39.15	-5.05	20
05-MC-065	1	31.38	44.64	-13.26	21
05-MC-063	1	39.04	33.22	5.82	22

Table 23. 120 Minute GEBT Y Group Data for Test Meal Equivalence Calculations

Experimental Data Y Group Data. Sequence B:A					
Subject #	**Stage**	**A(test) (kPCD)**	**B(ref) (kPCD)**	**Y=A-B (kPCD)**	**Count**
05-MC-021	1	46.25	46.95	-0.70	1
05-MC-022	1	66.98	61.01	5.97	2
05-MC-024	1	47.57	39.45	8.12	3
05-MC-027-R1	1	39.84	49.50	-9.66	4
05-MC-028-R1	1	53.23	52.33	0.90	5
05-MC-034	1	62.97	65.92	-2.95	6
05-MC-036	1	68.76	68.72	0.04	7
05-MC-037	1	70.56	68.23	2.33	8
05-MC-038	1	65.90	54.58	11.32	9
05-MC-040	1	82.38	78.86	3.52	10
05-MC-041	1	47.10	53.68	-6.58	11
05-MC-044-Rp2	1	55.30	70.67	-15.37	12
05-MC-045	1	62.37	58.22	4.15	13
05-MC-048	1	52.56	46.82	5.74	14
05-MC-050	1	52.60	45.44	7.16	15
05-MC-053	1	47.34	49.42	-2.08	16
05-MC-054	1	60.18	51.40	8.78	17
05-MC-056	1	51.23	47.96	3.27	18
05-MC-059	1	65.68	71.10	-5.42	19
05-MC-060	1	62.52	49.16	13.36	20
05-MC-062-R1	1	39.31	55.78	-16.47	21
05-MC-064	1	57.40	56.93	0.47	22

Using the δ (margin of equivalence limit) of ± 3.0 kPCD, the calculated experimental design limits for accepting or rejecting GEBT test meal equivalence at the 120 minute measurement time, after Stage 1, are as follows:

Experimental Design				
δ = 3.0			Acceptance Limit	Rejection Limit
Stage	**Nx_k**	**Ny_k**	**T^+_k**	**T^+_k**
1	22	22	-1.363	0.704

Using the experimental data from Stage 1 at 120 minutes, the calculated values met the criteria for GEBT equivalence at 120 minutes as shown below.

Results (Observed)			
Stage	**T^+_{obs}**	**T^-_{obs}**	**Action**
1	-2.379	2.280	**Accept Equivalence**

That is, the GEBT study data and calculations for the 120 minute measurement time point satisfied the following statistical criteria.

If the observed T^{+}_{k} < the Stage 1 acceptance limit of - 1.363
AND
If the observed T^{-}_{k} > - (the Stage 1 acceptance limit of 0.704)
THEN ACCEPT EQUIVALENCE.

Conclusion: GEBT test meals prepared by the Modified Process (MP) and the Original Process (OP) demonstrate statistically equivalent GEBT (kPCD) metrics.

11.4.2 Statistical/Analytical Issues

No statistical/analytical issues were encountered during this study.

11.4.3 Tabulation of Individual Response Data

The GEBT kPCD data for each study subject at each measurement time point for each meal administered (MP and OP) are provided in Tables 15.2-18, 15.2-23, 15.2-28 and 15.2-33 of Listing 15.2.8 in Appendix 15.2.

11.4.4 Analytical Performance Conclusions

When using the same dose of exogenous ^{13}C, 43 mg, supplied by ^{13}C-*Spirulina* Drug Substance produced by the intended commercial process, GEBT test meals prepared by the Modified Manufacturing Process (MP) demonstrate in vivo scintigraphic and GEBT metrics statistically equivalent to those derived from GEBT test meals prepared by the Original Manufacturing Process (OP).

SECTION 12 SAFETY EVALUATION

12.1 Adverse Events

12.1.1 Brief Summary of Adverse Events

Three adverse events (AEs) were reported in this study. Dr. Lawrence Szarka, PI, determined that none of the AEs was related to the GEBT or dual-label test procedure. There were no serious adverse events. A complete description of each AE, any actions taken, and other pertinent information are provided in Sections 12.1.2 and 12.1.3.

12.1.2 Display of Adverse Events

Table 24. Study PRO-CD-005 Adverse Events

Adverse Events (AEs)										
Subject ID	**AE Number**	**Date of Onset**	**Date Resolved**	**SAE?**	**Brief Description**	**AE Severity**	**AE Relationship to Study Device**	**AE Causality**	**Actions Taken**	**Outcome**
05-MC-030	1	09/29/2009	Unknown	No	Upper respiratory illness, congested nose, and fatigue reported 4 days after Visit 2 (first test meal)	Mild	Not Related	Concurrent illness: upper respiratory tract infection	None	Uncertain, patient not responsive to follow-up
05-MC-061	1	01/09/2010	01/18/2010	No	Upper respiratory infection with fever reported 1 day after Visit 2 (first test meal)	Moderate	Not Related	Concurrent illness: upper respiratory tract infection	Subject withdrew from study	Resolved with treatment
05-MC-061-Rp2*	1	02/05/2010	02/08/2010	No	Moderate nausea, dizziness, and low-grade fever reported at Visit 2 (first test meal)	Moderate	Not Related	Concurrent illness: suspected intercurrent infection	Subject withdrew from study	Resolved without treatment

* Rp means test subject was replacement for original subject. Rp1 is the first replacement subject, Rp2 the second replacement, etc.

12.1.3 Analysis of Adverse Events

Subject 05-MC-030 reported an upper respiratory infection with congested nose and fatigue on 10/01/2009 at study visit 3, second test meal administration and XXX days after Visit 2, the first test meal administration. Dr. Lawrence Szarka, study PI, determined that the reported adverse event (AE) was not related to the study device. No action was taken, and the subject completed the study at Visit 3. The Study Coordinator attempted to follow-up with the subject to determine how/when the AE was resolved, but the subject was unresponsive to follow-up attempts.

Subject 05-MC-061 reported an upper respiratory infection with fever on 01/09/2010, 2 days following the first test meal administration at study visit 2. Dr. Szarka determined that the reported AE was unrelated to the study device due to the subject being diagnosed with an upper respiratory tract infection, which was being treated with cephalexin 500 mg, twice daily for 10 days. The subject withdrew from the study and reported that the AE had resolved on 01/18/2010.

On 02/05/2010, at study visit 2 (first test meal administration), subject 05-MC-061-Rp2 reported nausea, dizziness, and a low-grade fever. Dr. Szarka suspected an intercurrent infection and determined that the AE was unrelated to the study device, as the subject had not received either test meal. No action was taken and the subject withdrew from the study. The subject reported that the AE resolved without treatment on 02/08/2010.

Note: an Rp signifier indicates that a subject was a replacement for the original subject assigned that subject ID. Subject 05-MC-061-Rp2 was the second replacement for original subject 05-MC-061.

12.1.4 Listing of Adverse Events by Test Subject

See Table 24, section 12.1.2 of this report.

12.2 Serious Adverse Events

No serious adverse events were reported by any subject in this study.

12.3 Vital Signs, Physical Findings and Other Observations Related to Safety

Vital signs measurements and physical findings were collected at Visits 2 and 3, prior to each administration of the test meal to each subject; there were no atypical results among vital signs measurements or physical findings in any subject who participated in this study. Vital signs data are available in Tables 15.2-12 and 15.2-13 of Listing 15.2.4 in Appendix 15.2.

12.4 Safety Conclusions

As no serious adverse events, or adverse events related to the study device, were reported by a total of 58 individual normal subjects who received the test meal on two separate occasions in this study, the results suggest that the test meal is safe for administration to healthy normal adults.

SECTION 13 DISCUSSION AND OVERALL CONCLUSIONS

The study demonstrated by objective evidence that the GEBT test meal produced by the proposed modified manufacturing process (MP) is equivalent to that produced by the original manufacturing process (OP) for use with gastric scintigraphy and GEBT methods for evaluation of gastric emptying in adults.

Test Meal Equivalence as Assessed by Scintigraphy

Using a δ (margin of equivalence limit) of 6.6 minutes, the calculated experimental design limits for accepting or rejecting scintigraphic test meal equivalence after Stage 1 were as follows:

Experimental Design				
δ = 6.6			Acceptance Limit	Rejection Limit
Stage	Nx_k	Ny_k	T^+_k	T^+_k
1	10	10	-1.395	0.713

Using the experimental data from 20 subjects tested by scintigraphy, the calculated values met the criteria for equivalence as shown below.

Results (Observed)			
Stage	T^+_{obs}	T^-_{obs}	Action
1	-4.319	1.775	Accept Equivalence

That is, the study data and calculations satisfied the statistical criteria that:

If the observed T^+_k < the Stage 1 acceptance limit of -1.395
AND
If the observed T^-_k > - (the Stage 1 acceptance limit of 0.713)
THEN ACCEPT EQUIVALENCE.

Conclusion: GEBT test meals prepared by the Modified Process (MP) and the Original Process (OP) empty from the human stomach of adults at equivalent rates as measured by gastric scintigraphy.

Test Meal Equivalence as Assessed by GEBT

The mean kPCD values for the respective OP and MP meals administered to the 44 GEBT test subjects in Stage 1 are shown in Table 25. Although the 90 and 120 minute measurement times are the critical times for calculating equivalence statistics, the average GEBT (kPCD) values for MP and OP are very close at all measurement times.

Table 25. Mean GEBT (kPCD) Values for OP and MP Test Meals: N=44

Measurement Time	45 Minutes	90 Minutes	120 Minutes	150 Minutes	180 Minutes
Mean: MP Meal	19.57	43.51	55.21	59.17	58.92
Mean: OP Meal	21.25	44.10	55.28	59.19	58.38
Average Δ (MP-OP)	-1.68	-0.59	-0.07	-0.02	0.54

The average intra-patient difference (Δ) in MP vs. OP meals was only 0.59 and 0.07 kPCD at the critical 90 and 120 minute measurement times, respectively. This is significantly less than the specified δ (margin of equivalence defined for GEBT) of ± 3.0 kPCD.

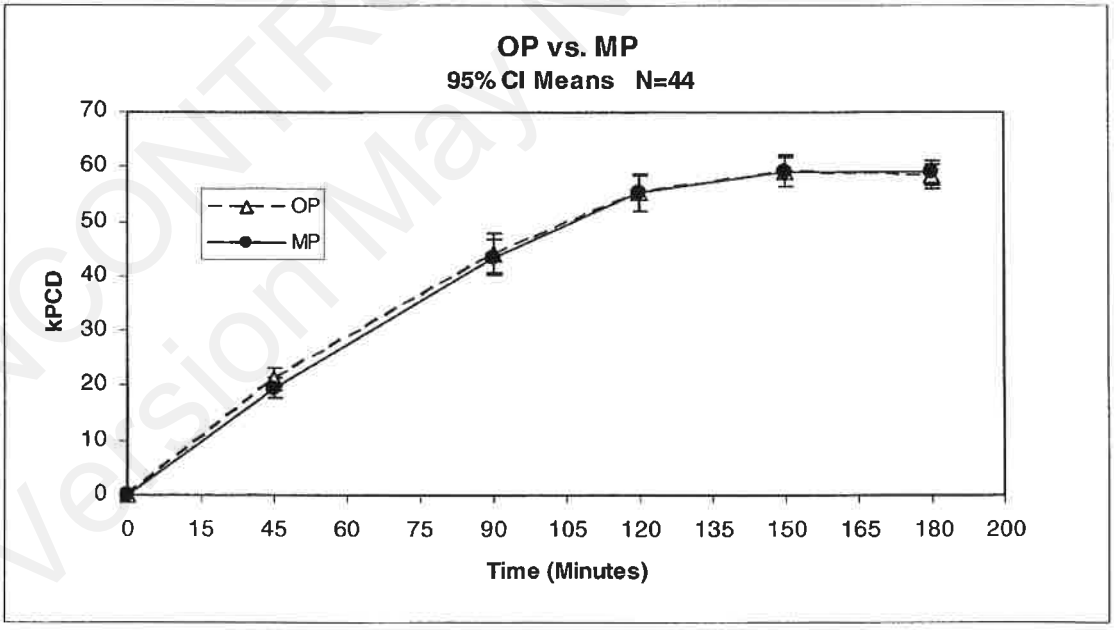
Also, the inter-subject standard deviation observed for MP and OP meals was very close at each measurement time, as presented in Table 26.

Table 26. Standard Deviations (SD) Observed for Respective MP and OP Meals: N=44

Measurement Time	45 Minutes	90 Minutes	120 Minutes	150 Minutes	180 Minutes
SD: MP Meal	6.38	11.10	11.50	9.56	7.69
SD: OP Meal	7.11	11.83	11.05	9.12	7.54
SD: Δ (MP-OP)	6.39	8.54	8.48	7.42	6.75

Figure 5 demonstrates the virtual equivalence of the two meals.

Figure 5. Mean Cumulative kPCD Values for OP vs. MP Test Meals



Utilizing the GEBT (kPCD) data from the 44 subjects at the 90 and 120 minute measurement times, calculations necessary to execute the two-sided test of equivalence required to declare meal equivalence were performed as described in PRO-CD-005-03, Sections 5(A) and 5(F) (see Appendix 15.1, Section 15.1.1), and in Section 9.6.1 of this report. The exact same calculations as those conducted for assessing scintigraphic meal equivalence were conducted for the GEBT at both the 90 and 120 minute time points.

Using a δ (margin of equivalence limit) of ± 3.0 kPCD, the calculated experimental design limits for accepting or rejecting GEBT test meal equivalence at the 90 minute measurement time, after Stage 1, are as follows:

Experimental Design				
δ = 3.0			Acceptance Limit	Rejection Limit
Stage	Nx_k	Ny_k	T^+_k	T^+_k
1	22	22	-1.363	0.704

Using the experimental data from Stage 1, the calculated values met the criteria for GEBT equivalence at 90 minutes as shown below.

Results (Observed)			
Stage	T^+_{obs}	T^-_{obs}	Action
1	-2.765	1.857	Accept Equivalence

That is, the GEBT study data and calculations for the 90 minute measurement time point satisfied the following statistical criteria.

If the observed T^+_k < the Stage 1 acceptance limit of -1.363
AND
If the observed T^-_k > - (the Stage 1 acceptance limit of 0.704)
THEN ACCEPT EQUIVALENCE.

Using the δ (margin of equivalence limit) of ± 3.0 kPCD, the calculated experimental design limits for accepting or rejecting GEBT test meal equivalence at the 120 minute measurement time, after Stage 1, are as follows:

Experimental Design				
δ = 3.0			Acceptance Limit	Rejection Limit
Stage	Nx_k	Ny_k	T^+_k	T^+_k
1	22	22	-1.363	0.704

Using the experimental data from Stage 1 at 120 minutes, the calculated values met the criteria for GEBT equivalence at 120 minutes as shown below.

Results (Observed)			
Stage	T^+_{obs}	T^-_{obs}	Action
1	-2.379	2.280	Accept Equivalence

That is, the GEBT study data and calculations for the 120 minute measurement time point satisfied the following statistical criteria.

If the observed T^{+}_{k} < the Stage 1 acceptance limit of - 1.363
AND
If the observed T^{-}_{k} > - (the Stage 1 acceptance limit of 0.704)
THEN ACCEPT EQUIVALENCE.

Conclusion: GEBT test meals prepared by the Modified Process (MP) and the Original Process (OP) demonstrate statistically equivalent GEBT (kPCD) metrics.

SECTION 14 REFERENCE LIST

See Appendix 15.4 for copies of the following publications referenced in this report.

1. Ciferri O. Spirulina the Edible Microorganism. *Microbiol Rev.* Dec. 1983: 551-578.

2. Spirulina. FDA Talk Paper. June 23, 1981.

3. Chamorro GA, et al. Pharmacology and toxicology of spirulina alga. *Rev Invest Clin.* 1996; 48(5): 389-99.

4. Salazar M, et al. Subchronic toxicity study in rats fed spirulina. *J Ethnopharmacol.* 1998; 62: 235-241.

5. Yoshino Y, et al. The chronic intoxication test of spirulina product fed to wistar rats. *Jpn J Nutr.* 1980; 38(4): 221-226.
(Note: Abstract provided in English, full article only available in Japanese.)

6. Salazar M, et al. Effect of spirulina maxima consumption on reproduction and peri- and postnatal development in rats. *Food Chem Toxicol.* 1996; 34: 353-359.

7. Krishnakumari MK, et al. Food safety evaluation: acute oral and dermal effects of the algae scenedesmus acutus and spirulina platensis on albino rats. *J Food Protect.* 1981; 44(12): 934-935.

8. Lee JS, et al. A valid, accurate, office based non-radioactive test for gastric emptying of solids. *Gut.* 2000; 46: 768-773.

9. Viramontes BE, et al. Validation of a stable isotope gastric emptying test for normal, accelerated or delayed gastric emptying. *Neurogastroent Motil.* 2001; 13: 567-574.

10. Schofield WN. Predicting Basal Metabolic Rate, New Standards and Review of Previous Work. *Hum Nutr-Clin Nutr.* 1985; 39(C)(suppl 1): 5-41.

11. Jennison C, Turnbull BW. *Group Sequential Methods with Applications to Clinical Trials.* Boca Raton, FL: Chapman and Hall/CRC; 2000: 111-144.
(Note: Copy not provided, available upon request.)

12. Klein PD, et al. Normalizing Results of 13C-Urea Breath Testing for CO2 Production Rates. *J Pediatr Gastr Nutr.* 1999; 29: 297-301.

SECTION 15 APPENDICES

This section contains the following appendices, which respectively contain the specified information.

Appendix 15.1, Study Information, contains the following information; detailed appendix contents are provided on the first page (15.1-1) of Appendix 15.1.

1. Study Protocol
2. GEBT Administration Instructions
3. Sample Case Report Forms
4. IRB Information
5. Sample Consent Forms
6. List and Description of Investigators
7. List of Test Subjects Receiving Investigational Product
8. Randomization Schemes
9. Individual Subject Test Kit Assignments
10. Monitoring Reports
11. Documentation of Statistical Methods
12. Documentation of Quality Assurance Procedures
13. Device Failures
14. Publications Based on the Study

Appendix 15.2, Patient Data Listings, contains the following listings; detailed appendix contents are provided on the first page (15.2-1) of Appendix 15.2.

1. Discontinued Patients
2. Protocol Deviations
3. Patients Excluded from Analysis
4. Baseline Data, i.e., demographic information, test dates, medical histories, etc.
5. Drug Concentration Data
6. Individual Adverse Event Listings
7. Individual Vital Signs Measurements
8. Individual Test Subject Data Listings, i.e., efficacy data

Appendix 15.3, Case Report Forms, contains a copy of the case report forms of serious adverse events and withdrawals for adverse events.

Appendix 15.4 contains copies of publications referenced in this report.

22.0 EXHIBIT C: COVID-19 TRANSMISSION MITIGATION PLAN

COVID-19 TRANSMISSION MITIGATION PLAN

Visit 1: Screening visit

Cairn personnel designated to participate in screening visit with the potential study participant will be required to wear a facemask and practice social distancing while in contact with the potential participant.

The potential study participant will enter the facility where they will be met by Cairn personnel and will be asked a series of questions regarding symptomology or exposure to COVID-19 (refer to Exhibit E for Study Participant Health Questionnaire). A copy of the questionnaire will be signed by the participant and Cairn personnel and will be stored in the participants file.

- If participant answers any of the questions that indicate potential symptomology or exposure to COVID-19, they will be asked to leave the facility and be advised to consult a healthcare professional. They will not be able to participate in the study at that time.
- If the participant answers the questions indicating no potential symptomology or no known exposure to COVID-19, they will meet with a qualified Cairn employee designated by the medical director and/or the medical director to give an overview explanation of the study, answer questions regarding the GEBT test procedure and provide consent to participate in the study.

Upon completion of this screening visit, the study participant will leave the facility and all PPEs worn by Cairn personnel will be disposed of in a biohazard container.

Visits 2 and 3: GEBT Administration

Cairn personnel designated to participate in GEBT administration with the study participant will be required to wear a disposable laboratory coat, gloves and facemask while in contact with the potential participant.

Cairn personnel designated to handle the participant's breath bag samples and transferring to multiple vacutainer tubes will be required to wear gloves and facemask while in contact with the samples.

The study participant will enter the facility where they will be met by Cairn personnel and will be asked a series of questions regarding symptomology or exposure to COVID-19 (refer to Exhibit E for Study Participant Health Questionnaire). A copy of the questionnaire will be signed by the participant and Cairn personnel and will be stored in the participants file.

- If participant answers any of the questions that indicate potential symptomology or exposure to COVID-19, they will be asked to leave the facility and be advised to consult a healthcare professional. They will not be able to participate in the study at that time.
- If the participant answers the questions indicating no potential symptomology or no known exposure to COVID-19, they will meet with the a qualified Cairn employee designated by the medical director and/or the medical director to give an overview explanation of the study, answer questions regarding the GEBT test procedure and provide consent to participate in the study.

The participant will be administered the GEBT under the supervision of Cairn's medical director via a telecommunication platform (e.g. FaceTime, Microsoft Teams, etc) by qualified Cairn personnel. The participant will remain in the area where the GEBT is being administered and will only be in contact with the qualified personnel during sample collection times.

Upon completion of the GEBT administration visit, the study participant will leave the facility and all PPEs worn by Cairn personnel will be disposed of in a biohazard container.

Visit 4: Follow up visit

Cairn personnel designated to participate in follow up contact with the participant will do so via a phone call, email, or a telecommunication platform. If the follow up if performed via a phone call or telecommunication platform, a memo noting the follow up will be placed in the participant's file.

23.0 EXHIBIT D: STUDY PARTICIPANT HEALTH QUESTIONNAIRE

Study Participant Health Questionnaire

In order to prevent the spread of COVID-19 (novel coronavirus) and reduce the exposure of our personnel, their families, and our visitors, we would appreciate you completing the following health questionnaire.

1) Do you have a fever currently or in the past 14 days? ☐ **Yes** ☐ **No**

2) Are you currently experiencing a cough, sore throat, shortness of breath, muscle aches/pains, or diarrhea? ☐ **Yes** ☐ **No**

3) Have you had contact with anyone who has confirmed, suspected, or has been tested and is awaiting results for COVID-19? ☐ **Yes** ☐ **No**

4) Have you been tested for COVID-19? ☐ **Yes** ☐ **No**

   a. If yes, when and results: _____

_____ _____
Study Participant Signature Date

_____ _____
Cairn Personnel Signature Date

24.0 EXHIBIT E: CONTRACEPTIVE GUIDANCE AND COLLECTION OF PREGNANCY INFORMATION

Woman of Childbearing Potential (WOCBP)

A woman is considered fertile following menarche and until becoming post-menopausal unless permanently sterile (see below).

Women in the following categories are not considered WOCBP

1. Premenarchal
2. Premenopausal female with 1 of the following:
   - Documented hysterectomy
   - Documented bilateral salpingectomy
   - Documented bilateral oophorectomy

Note: Documentation can come from the site personnel's: review of the participant's medical records, medical examination, or medical history interview.

3. Postmenopausal female

   - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormonal replacement therapy (HRT). However, in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.
   - Females on HRT and whose menopausal status is in doubt will be required to use one of the non-estrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Contraception Guidance:

Female participants

Female participants of childbearing potential are eligible to participate if they agree to use a highly effective method of contraception consistently and correctly as described in Table 1.

Table 1: Highly Effective Contraceptive Methods

Highly Effective Contraceptive Methods That Are User Dependent[a] *Failure rate of <1% per year when used consistently and correctly.*
Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation[b] • Oral • Intravaginal • Transdermal
Progestogen only hormonal contraception associated with inhibition of ovulation • Oral • Injectable

Highly Effective Methods That Are User Independent [a]
Implantable progestogen only hormonal contraception associated with inhibition of ovulation[b] • Intrauterine device (IUD) • Intrauterine hormone-releasing system (IUS) • Bilateral tubal occlusion
Vasectomized partner *A vasectomized partner is a highly effective contraception method provided that the partner is the sole male sexual partner of the WOCBP and the absence of sperm has been confirmed. If not, an additional highly effective method of contraception should be used.*
Sexual abstinence *Sexual abstinence is considered a highly effective method only if defined as refraining from heterosexual intercourse during the entire period of risk associated with the study intervention. The reliability of sexual abstinence needs to be evaluated in relation to the duration of the study and the preferred and usual lifestyle of the participant.*
NOTES: a) Typical use failure rates may differ from those when used consistently and correctly. Use should be consistent with local regulations regarding the use of contraceptive methods for participants participating in clinical studies. b) There is no scientific reason to believe that ^{13}C-Spirulina GEBT will interact with hormonal contraception and reduce the efficacy of the contraceptive method.

Pregnancy Testing:

- WOCBP should only be included after a confirmed menstrual period and a negative highly sensitive urine or serum pregnancy test.
- Perform pregnancy testing with a minimum sensitivity of 25 mIU/mL on WOCBP within 48 hours prior to each Gastric Emptying Breath Test.
- Pregnancy testing will be performed whenever a menstrual cycle is missed or when pregnancy is otherwise suspected.

Collection of Pregnancy Information:

Female Participants who become pregnant

- The investigator will collect pregnancy information on any female participant who becomes pregnant while participating in this study. Information will be recorded on the appropriate form and submitted to the sponsor within 24 hours of learning of a participant's pregnancy. The participant will be followed to determine the outcome of the pregnancy. The investigator will collect follow-up information on the participant and the neonate and the information will be forwarded to the sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.
- While pregnancy itself is not considered to be an AE or SAE, and while there are not expected to be any adverse effects of ^{13}C-Spirulina GEBT on a pregnancy, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE. A spontaneous abortion is always considered to be an SAE and will be reported as such. Any post-study pregnancy related SAE considered reasonably related to the study intervention

by the investigator will be reported to the sponsor as described in Section 8.4. While the investigator is not obligated to actively seek this information in former study participants, he or she may learn of an SAE through spontaneous reporting.

Any female participant who becomes pregnant while participating in the study will be withdrawn from the study.