^{13}C-Spirulina Gastric Emptying Breath Test Kit "GEBT"

1. Intended Use IVD

The Gastric Emptying Breath Test (GEBT), 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 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 procedure should be administered under supervision of a health care professional although no specialized facilities or specially licensed personnel are required.

2. Summary and Explanation

2.1 Description of Gastroparesis and Patient Populations(s)

Gastroparesis (delayed gastric emptying) is a common cause of nausea, vomiting, early satiety, postprandial fullness and other upper gut symptoms in individuals referred to gastroenterologists.[1, 2] Gastroparesis is a form of gastric paralysis causing significant morbidity.[3] Weight loss, malnutrition and dehydration may be prominent in severe cases and may require tertiary care.[2, 3, 4] While the true prevalence of gastroparesis is unknown, it is estimated to occur in 20% - 40% of individuals with diabetes mellitus, particularly type I diabetes mellitus, and may be present in 25% - 40% of individuals with functional (non-ulcer) dyspepsia, a condition affecting approximately 20% of the US general population.[1] In diabetics, gastroparesis may adversely affect glycemic control. The etiology of gastroparesis is diverse; the main categories are diabetic, idiopathic and post-surgical.[1, 2, 3, 4] Virtually any disease or condition that can induce neuromuscular dysfunction of the gastrointestinal tract may cause gastroparesis.[3]

Generally, there is only weak correlation between symptom severity and the degree of gastric stasis in gastroparetics. In diabetics, the correlation between global gastric symptoms and rates of gastric emptying is poor. Gastroparesis is typically diagnosed based on the presence of appropriate symptoms/signs, confirmation of delayed gastric emptying, and the absence of an obstructing structural lesion in the stomach or small intestine.[1, 2, 3, 4]

Symptoms of gastroparesis are not significantly dissimilar to symptoms characteristic of various GI conditions (e.g., functional dyspepsia, gastroesophageal reflux disease GERD, dumping syndrome, peptic ulcer disease, etc.). Hence, assessment of gastric motility can be important for the purpose of differential diagnosis and proper treatment planning in patients symptomatic for or suspected of having gastroparesis. Management of delayed gastric emptying is focused on dietary changes, nutritional support, pharmacologic intervention with antiemetic and prokinetic agents in most

individuals, and correction of glycemic control in diabetics. Individuals who are unresponsive to initial treatment can be difficult to manage and may require switching and/or combining of prokinetic and antiemetic agents. Endoscopic venting and feeding tubes utilized in accordance with symptoms and nutritional requirements result in reduced need for hospitalization with refractory gastroparesis. Evolving treatments have been used in some difficult cases, including injecting botulinum toxin in the pylorus and implanting a gastric electric stimulator.[1, 2, 3, 4]

2.2 GEBT as a Surrogate Test for Gastric Scintigraphy

The GEBT is a non-radioactive, non-invasive, orally administered test for measurement of the rate of solid phase gastric emptying in adults. The GEBT has been validated against the gold standard reference method of gastric scintigraphy.[5] Performance characteristics of the GEBT compared to gastric scintigraphy are reported in Section 11. The GEBT may be administered in a primary care facility, clinic or tertiary care setting.

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

3. Description/Principle of the GEBT

3.1 Description of ^{13}C-Spirulina Diagnostic Substrate

The GEBT is a non-radioactive breath test utilizing the stable isotope carbon-13, denoted as ^{13}C. ^{13}C stable isotope labeling is inherently safe as 1.1% of all the carbon in nature, our bodies and in the food we eat is ^{13}C, with the remaining 98.9% being ^{12}C.

Spirulina is a safe, edible, highly digestible blue-green microalga. In the US, Spirulina is primarily consumed as a health food due to its rich nutritional qualities. The recommended amount of Spirulina as a dietary supplement ranges from 3 to 4.5 grams per day. The dose of ^{13}C-Spirulina in each GEBT meal is approximately 0.1 grams. Spirulina was acknowledged as a "legally marketed" food by the US FDA in 1981.[6] In 2011 the Dietary Supplements Information Expert Committee (DSI-EC) of the United States Pharmacopeia (USP) performed a comprehensive review of information related to safety and toxicology of Spirulina and subsequently unanimously voted to grant Class A safety assignment (i.e., "available evidence does not indicate a serious risk to health or other public health concern that precludes admission of quality monographs into US-NF when these dietary ingredients are properly identified, formulated, and used") to *Spirulina platensis* and *Spirulina maxima*.[7] Based on this decision, USP has verified Spirulina as a dietary ingredient and is currently developing a quality monograph for Spirulina.[7]

The ^{13}C-Spirulina used in the GEBT meal is cultured in a >95% ^{13}C enriched medium, resulting in full ^{13}C-labeling of nearly all of the algae's carbon containing nutrients. During GEBT administration, ^{13}C-Spirulina particles are bound to the re-hydrated, cooked egg meal and travel with the solid phase of the test meal. The ^{13}C-label is

released after the GEBT meal exits the stomach during digestion. In the upper small intestine, the absorption of ^{13}C-Spirulina follows the same pathways as the absorption of other macronutrients.

3.2 Principle of the GEBT

After providing duplicate pre-meal breath samples, the individual being tested consumes the standard GEBT meal consisting of 27 grams of re-hydrated, pasteurized scrambled egg mix containing a dose of 43 mg of ^{13}C (provided by approximately 100 mg of ^{13}C-Spirulina), 6 saltine crackers, and 6 fl oz (180 mL) of potable water. The caloric value of the meal is approximately 230 kCal. As the egg meal containing the ^{13}C-Spirulina is triturated by the stomach to a particle size of 1 – 2 mm, it passes through the pylorus into the intestine. In the intestine, the labeled products of ^{13}C-Spirulina digestion (proteins, carbohydrates, and fats) are absorbed and metabolized giving rise to ^{13}C-labeled carbon dioxide expired in the breath. Breath samples, collected periodically in capped glass tubes before and after test meal administration, are returned to a central laboratory for analysis by Gas Isotope Ratio Mass Spectrometry (GIRMS) to determine the ratio of $^{13}CO_2/^{12}CO_2$ in each sample. By measuring the change in this ratio over time as compared to the pre-meal value, the rate of $^{13}CO_2$ excretion can be calculated and the individual's gastric emptying rate determined.

3.3 GEBT Configuration

The GEBT procedure is conducted over a 4 hour period. Following an overnight (or ≥8 hour) fast, duplicate pre-meal breath samples are collected from the test subject. Two pre-meal samples are collected to ensure that in the event of breakage, leakage or a lost sample, there is a "back-up" available in order to establish a subject's baseline $^{13}CO_2$ level. The first intact sample of the two pre-meal samples will be analyzed to establish the subject's baseline $^{13}CO_2$ value. Following pre-meal sample collection, the subject is administered the test meal. Single post-meal breath samples are subsequently collected at 45, 90, 120, 150, 180, and 240 minutes from the end of test meal consumption.

3.4 Attributes of the GEBT Meal

The emptying time of the GEBT meal was determined by scintigraphy and is similar to low-fat egg meals utilized with scintigraphy. Table 1 displays the average fraction (%) of GEBT meal retained in the stomach of healthy subjects over the 4-hour GEBT evaluation period.

Table 1. Scintigraphic Fraction of GEBT Meal Retained (N=30 healthy subjects)

Measurement Time	0 Min	45 Min	90 Min	120 Min	150 Min	180 Min	240 Min
% Test Meal Retained	100	66	34	18	9	4	1

By contrast, Table 2 displays the average fraction (%) of test meal retained in delayed subjects over the 4-hour GEBT evaluation period.

Table 2. Scintigraphic Fraction of GEBT Meal Retained (N=57 delayed subjects*)

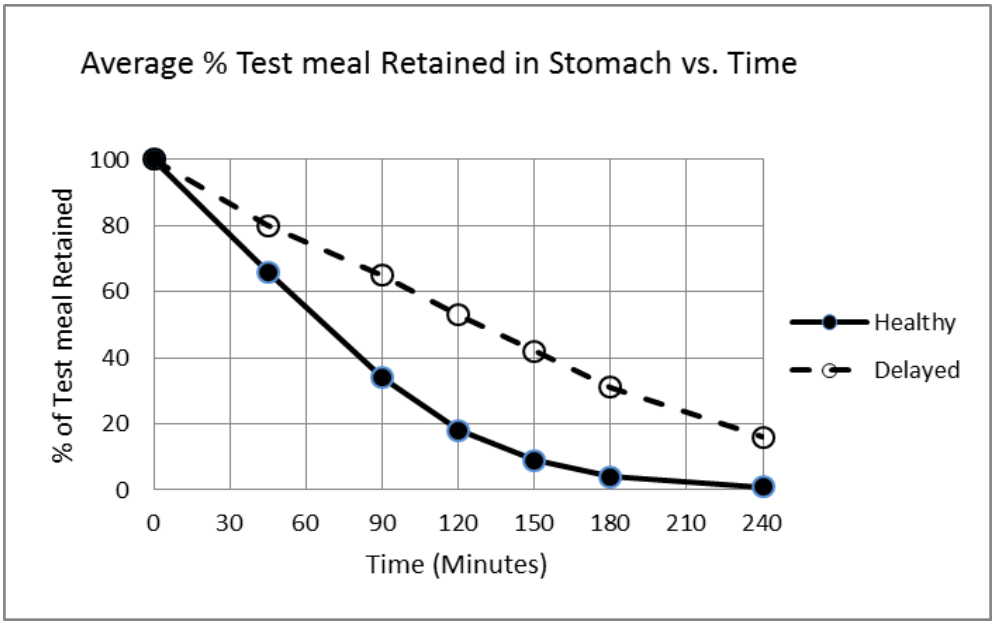
Measurement Time	0 Min	45 Min	90 Min	120 Min	150 Min	180 Min	240 Min
% Test Meal Retained	100	80	65	53	42	31	16

*Scintigraphically delayed subjects who participated in ABD validation study PRO-CD-004

Figure 1 graphically contrasts the average scintigraphic emptying profiles of healthy vs. delayed (gastroparetic) subjects administered the GEBT meal.

Utilizing a GEBT meal containing 200 mg of ^{13}C-Spirulina (twice the carbon-13 dose in the standardized GEBT meal), the carbon-13 washout period was assessed and determined to be 24 hours. Hence, the GEBT may be administered as often as every 24 hours.

Figure 1. Average Fraction of Test Meal Retained in the Stomach vs. Time (as Measured by Scintigraphy)



The normal, inherent, day-to-day, within-subject biologic variability of gastric emptying is substantial. Biologic variability was assessed by GEBT and scintigraphy in patients with normal gastric emptying and patients with slightly (marginally) delayed

gastric emptying. Table 3 displays the standard deviation (SD) and % coefficient of variation (%CV) of within-subject biologic variability (BV) at each measurement time for each respective test method using the GEBT meal. Each test subject was given the standardized GEBT twice; test administration was conducted on independent days. Within-subject biologic variability is similar when measured by either test method (GEBT vs. scintigraphy).

Table 3. Within-Subject Biologic Variability (BV) of Gastric Emptying

Procedure	Class	N	Measurement Time in minutes, measured from the end of test meal ingestion					
			45	90	120	150	180	240
GEBT $(SD)_{BV}$	Normal	25	4.9	7.4	6.7	5.0	4.8	3.60
GEBT $(\%CV)_{BV}$			19.9	15.3	11.5	8.2	8.0	7.2
Scintigraphy $(SD)_{BV}$		20	7.2	8.0	7.1	6.3	4.9	1.8*
Scintigraphy $(\%CV)_{BV}$			20.50	12.20	8.50	5.50	3.60	1.7*
GEBT $(SD)_{BV}$	Marginally Delayed	8	2.9	5.0	5.3	5.8	5.0	2.2
GEBT $(\%CV)_{BV}$			12.8	11.9	10.3	10.4	8.7	3.9
Scintigraphy $(SD)_{BV}$		8	8.2	6.5	7.4	8.9	7.5	5.5
Scintigraphy $(\%CV)_{BV}$			24.7	11.3	10.2	11.0	8.4	5.6

*The SD and %CV is low as the stomach is nearly emptied in healthy subjects at this time point

3.5 List of all materials provided with the GEBT kit:

- 3.5.1 1 Pouch of Diagnostic Dosage (^{13}C-Spirulina/Egg Mix in a foil pouch with oxygen absorber)
- 3.5.2 6 Saltine crackers (3 packages, of two crackers each, repackaged in a foil pouch with oxygen absorber)
- 3.5.3 1 large (~13 fl oz/390 mL) microwaveable cooking cup
- 3.5.4 1 filling cup (small (~3.5 fl oz/~100 mL) plastic cup with pour spout for transferring water)
- 3.5.5 1 plastic cutlery kit (knife, fork and spoon)
- 3.5.6 2 wrapped, plastic straws
- 3.5.7 8 barcode labeled, glass, breath collection tubes with screw caps
- 3.5.8 1 breath tube holder
- 3.5.9 1 mailer
- 3.5.10 1 GEBT requisition form
- 3.5.11 1 package insert

3.6 List of items needed to perform the GEBT, but not provided with the GEBT Kit:

- 3.6.1 Interval timer or clock.
- 3.6.2 Disposable drinking cup capable of holding 6 fl oz (180 mL) of water.
- 3.6.3 1 cu. Ft. (0.02 m^3), 1100-watt microwave oven with turntable.
- 3.6.4 Disposable plate.

4. Contraindications

4.1 Individuals with known hypersensitivity to Spirulina, egg, milk or wheat allergens should avoid the GEBT.

4.2 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 bowel malabsorption.

5. Warnings and Precautions

5.1 For in vitro diagnostic use only. The GEBT meal containing the diagnostic substrate ^{13}C-Spirulina is consumed orally as part of the GEBT diagnostic procedure.

5.2 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.

5.3 The performance characteristics for individuals under the age of eighteen (18) years have not been established for this test.

5.4 The performance characteristics for pregnant women have not been established for this test.

5.5 False positive and false negative results can occur with this test.

5.6 Follow the directions for collecting breath samples carefully. Errors in the timing and/or procedure for collecting breath samples may affect test results and necessitate re-testing.

5.7 This test 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.

5.8 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 <275 mg/dL[9].

5.9 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.

5.10 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*).

6. Adverse Events

In total, 321 adult subjects were administered the GEBT during development and validation studies. There were no deaths associated with GEBT studies. There were no serious adverse events associated with the GEBT device or the dual-label test procedure conducted in GEBT studies. There were 20 non-serious adverse events. Of these events, 15 were determined to be unrelated to the GEBT, four were determined to be of uncertain relation to the GEBT, and one was determined to be associated with the GEBT. All four events for which the relationship to the GEBT was considered uncertain were reported by subjects previously determined to be gastroparetic. Additionally, the one event that was determined to be related to the GEBT was reported by a gastroparetic subject. The five reporting subjects complained of nausea and abdominal discomfort after ingesting the test meals, which are symptoms consistent with gastroparesis, especially following food consumption.

GEBT has been authorized for use as an investigational device in investigations of promising pro-kinetic drugs conducted by sponsors other than ABD under IND approval. In these studies, over 1700 adult patients have been administered the GEBT. No adverse events related to or suspected of being related to the GEBT have been reported in these studies.

7. Shelf Life and Storage

The ^{13}C-GEBT Administration Kit should be stored at controlled room temperature*. The kit has an expiry date; do not use a GEBT Kit beyond the expiration date displayed on the kit box.

Collected breath samples should be stored at controlled room temperature*. Ship breath samples to Cairn Diagnostics, in the sample mailer, within three weeks of collection.

*Controlled room temperature: a temperature maintained thermostatically that encompasses the usual and customary working environment of 20^{o}C to 25^{o}C (68^{o}F to 77^{o}F).

8. Subject Preparation

The GEBT should be administered after an overnight fast. No solid food should be consumed or vigorous activity undertaken within 8 hours prior to the test. Alcohol should not be ingested within 8 hours prior to testing. The individual being tested may consume a small amount of water up to 1 hour before the test, but not more than 4 fl oz (120 mL). Coffee may enhance gastric motility and should not be consumed within 8 hours prior to testing. Subjects should not smoke/use tobacco products (e.g. chewing tobacco, nicotine gum) before or during administration of GEBT.

The test administrator should confirm that the individual being tested does not have a known hypersensitivity to Spirulina, egg, milk, or wheat allergens; is not severely lactose intolerant; and has not taken medications known to influence the rate of gastric emptying (e.g., erythromycin, metoclopramide, opiates and anticholinergics) within 3 days of testing.

For diabetic test subjects, a fasting serum glucose level should be obtained to confirm that the patient is in glycemic control as hyperglycemia may produce a clinically significant delay in gastric emptying[8]. Serum glucose should be <275 mg/dL prior to conducting a gastric emptying evaluation[9]. If serum glucose cannot be lowered with insulin to below 275 mg/dL, the GEBT procedure should be re-scheduled for another day when the subject is in glycemic control.

The GEBT procedure should be performed in a comfortable environment. The individual being tested should sit quietly, refrain from smoking, and fast (with the exception of the test meal) for the duration of the test. Limited walking is allowed, when necessary, e.g., use of restroom.

9. GEBT Administration Instructions

GEBT ADMINISTRATION INSTRUCTIONS

1. Confirm fasting requirements have been met: no solid food or liquids (including alcoholic beverages) for 8 hours, with the exception of 4 fl oz (120 mL) water allowed up to 1 hour before the test.
2. Confirm that the patient has not received another ^{13}C breath test within 24 hours and if the patient is diabetic, that their blood serum glucose <275mg/dL.
3. Patient should sit quietly, refrain from smoking and (with the exception of the test meal) fast for the duration of the test.
4. Enter the required information into GEBT Requisition Form. ALL patient demographic information is REQUIRED for calculation of test results.
5. Collect two PRE-MEAL breath samples according to the collection procedure described below.
6. Prepare GEBT meal:
    - Empty the contents of the GEBT Diagnostic Dosage pouch into the cooking cup and remove the oxygen absorber.
    - Using the graduated filling cup, add 3 fl oz (90 mL) potable water to the GEBT Diagnostic Dosage in the cooking cup to rehydrate.
    - Stir the GEBT Diagnostic Dosage with the spoon provided and cook for 60 seconds in a 1100 Watt microwave (mixture should not be allowed to rehydrate for longer than 5 minutes).
    - Flip the partially cooked GEBT Diagnostic Dosage with the spoon and cook for an additional 30 seconds.
    - CAUTION: cooked GEBT Diagnostic Dosage will be hot.
7. Serve the cooked GEBT Diagnostic Dosage, saltine crackers and 6 oz (180 mL) potable water to the patient. The meal must be consumed within 10 minutes (record time on GEBT requisition form).
8. Set a timer for post meal breath collection as soon as the patient has fully consumed the meal.
9. Collect post-meal breath samples at **45, 90, 120, 150, 180,** and **240** minutes from time of completion of the meal according to the collection procedure described below.
10. Return a copy of the completed GEBT requisition form and collected breath samples in the breath sample tube transport container to Cairn Diagnostics in the provided mailer.

BREATH SAMPLE COLLECTION PROCEDURE

1. Remove the cap from breath sample tube and insert straw in tube.
2. Instruct the patient to:
    - Hold tube in comfortable position
    - Take a deep breath and pause momentarily
    - Blow gently into the tube for 5-10 seconds
    - Slowly withdraw straw from tube while still blowing into tube
3. Immediately cap the tube securely (over tightening may cause the septum cap to collapse).
4. Tubes containing breath samples are easily identified by the presence of condensation inside the tube.

10. Quality Control

Breath sample analysis is conducted in a clinical reference laboratory by appropriately trained and licensed personnel. The reference laboratory follows applicable internal quality control procedures. Gas Isotope Ratio Mass Spectrometry (GIRMS) instrumentation used to determine the $^{13}CO_2$ to $^{12}CO_2$ ratio in breath is tuned daily to ensure quality control parameters are acceptable. To ensure analytical accuracy, quality control procedures are performed using quality control gases of known $^{13}CO_2/^{12}CO_2$ concentrations.

Properly collected breath samples contain between approximately 3% and 5% CO_2. Breath samples containing less than 1% CO_2 do not contain sufficient CO_2 for accurate determination of the ratio of $^{13}CO_2$ to $^{12}CO_2$ and may necessitate re-testing. Test administrators should follow the breath sample collection procedure described in Section 9 above very carefully, to ensure that breath samples can be accurately analyzed.

11. GEBT Results

11.1 The Test Method

Determination of the ratio of $^{13}CO_2$ to $^{12}CO_2$ in each breath sample is conducted by the method of Gas Isotope Ratio Mass Spectrometry (GIRMS) at the reference laboratory.

11.2 Calculation of Test Results

Calculation of a subject's GEBT results is performed by the reference laboratory and reported to the ordering physician. No calculations are required by the ordering physician or test administration personnel.

11.3 Reporting of Test Results

GEBT results are reported using kPCD, a metric which expresses a subject's $^{13}CO_2$ excretion rate at each measurement time as defined below.

> **kPCD**: a mathematical expression of a test subject's $^{13}CO_2$ excretion rate (per minute, denoted as min^{-1}) at any measurement time **t** relative to the dose of carbon-13 contained in the test meal.
>
> Simply stated:
> **$kPCD_{(t)}$** = 1,000 x [percent dose ^{13}C excreted as $^{13}CO_2$ per minute (min^{-1}) at time t]

GEBT reports include the following information:

1. kPCD results at each measurement time (i.e., $kPCD_{45}$, $kPCD_{90}$, etc)
2. Reference range cutoff points for each measurement time
3. Patient status at each time point (Delayed vs. Normal)
4. A graphical display of the patient's $^{13}CO_2$ excretion rate (kPCD) vs. time

11.4 GEBT Reference Range Cut-Off Points (COP)

Sixty (60) healthy individuals at two sites provided pre-meal breath samples, were administered the standardized GEBT meal and subsequently provided post-meal breath samples at 45, 90, 120, 150, 180 and 240 minutes from the end of test meal ingestion. All breath samples were analyzed by GIRMS and the breath test metric kPCD was calculated for each sample. The central 95% Reference Interval for the kPCD data set obtained at each respective measurement time was then calculated using RefVal, a computer program implementing the recommendations of the International Federation of Clinical Chemistry on the statistical treatment of reference values.[10] The lower limit of the 95% reference range was established as the cut-off point to diagnose delayed gastric emptying (gastroparesis) by kPCD metric at each time point (Table 4). When using the kPCD metric, the cut-off point (COP) demarcates delayed from normal gastric emptying. For example, at the 90 minute measurement time, a patient having a kPCD result <26.9 min^{-1} would be classified as positive for delayed emptying (gastroparetic).

Table 4. GEBT Reference Range Cut-Off Points

Time point	kPCD (min^{-1})
45 min	12.9
90 min	26.9
120 min	34.4
150 min	39.5
180 min	43.0
240 min	35.0

11.5 Interpretation of GEBT Results

The GEBT measures the rate of $^{13}CO_2$ excretion after consumption of a ^{13}C-enriched test meal. As the ^{13}C enriched meal is emptied from the stomach it is digested, assimilated and $^{13}CO_2$ is excreted in the breath. At any given measurement time *t*, the $^{13}CO_2$ excretion rate is related to the rate of gastric emptying. A larger kPCD value means a faster $^{13}CO_2$ excretion rate which is proportional to a faster rate of gastric emptying.

In subjects with normal gastric emptying, $^{13}CO_2$ excretion rates obtained by GEBT generally rise to a maximum between 120 and 180 minutes and then start to decrease because nearly all of the of the ^{13}C-labeled test meal has been emptied from the stomach. In subjects with delayed emptying, $^{13}CO_2$ excretion rates likewise start to rise after ingestion of the meal, but rates are lower and rarely reach a maximum before the end of the evaluation period (240 minutes) as a significant portion of the meal remains in the stomach and digestion and assimilation are incomplete. Because excretion curves (kPCD values) of subjects with normal emptying are typically declining after 180 minutes and those of delayed subjects are still rising, kPCD values of modestly delayed subjects may rise through the COP and into the "normal" emptying rate zone at the 180 and 240 minute time points. Consequently, with breath test, the earlier time points are more sensitive for diagnosing gastroparesis (See Section 12.3.1, Table 5).

Figure 2 displays typical $^{13}CO_2$ excretion curves (kPCD metric) of subjects with normal, borderline and delayed rates of gastric emptying. For comparison, Figure 3 shows scintigraphy results for the same patients displayed in Figure 2.

Figure 2. Discrimination between Normal, Delayed and Borderline Subjects by GEBT

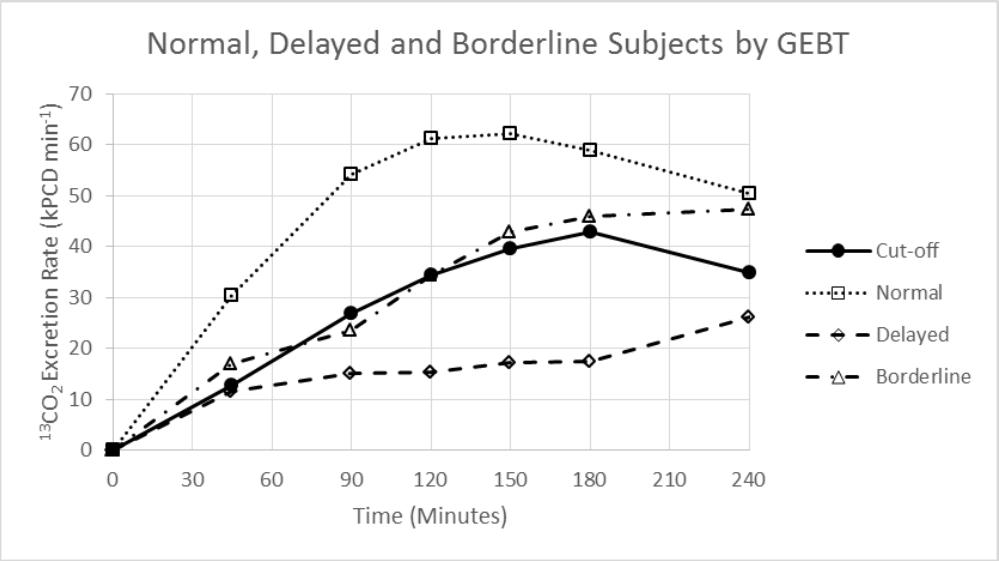
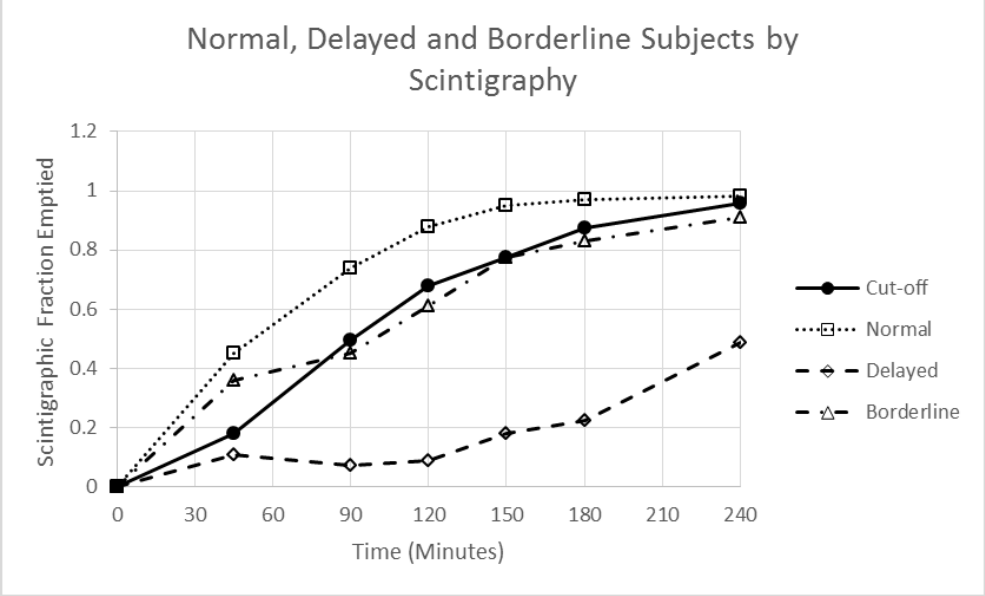


Figure 3. Discrimination between Normal, Delayed and Borderline Subjects by Scintigraphy



The contrasting shape of the excretion curves of normal vs. delayed subjects is informative. Although the curves in Figure 2 and 3 appear fairly similar, note that at later time points (180 and 240 minutes) the $^{13}CO_2$ excretion curve obtained by breath test from subjects with normal rates of gastric emptying declines while the scintigraphic curve does not. With breath test, in normal emptying subjects, the assimilated ^{13}C-label will take longer than 4 hours to entirely leave the body and will continuously decline with time, whereas with scintigraphy, physical emptying in normal subjects is observed to be entirely complete (or nearly so) at 4 hours (e.g., the fraction of test meal emptied

approaches 100% and cannot come down). However, note that in delayed subjects, emptying curves obtained by both test methods are similar; they are lower and generally continue to rise during the evaluation period.

As further demonstrated in Figures 2 and 3 above, some patients will have consistent test results across all time points (i.e., Normal at all time points, or Delayed at all time points) and are easily classified as having either a Normal or Delayed rate of gastric emptying.

However, as reported in the scintigraphic literature, a considerable number of symptomatic patients have rates of gastric emptying that reside close to the cut-off points (COPs) that demarcate Normal from Delayed emptying (i.e., borderline test results). In such patients, scintigraphic diagnostic classifications may conflict across measurement times. As with scintigraphy, diagnosis of delayed gastric emptying by GEBT may not be consistent across all time points for some patients.

GEBT results are intended to provide supplemental information to the clinician when evaluating a patient for gastroparesis. GEBT test results are best used as a component of an overall evaluation plan that includes the patient's symptomology (including such validated tools as PAGI-SYM/GCSI scores), clinical history, other laboratory test results and findings from procedures such as endoscopy. Diagnosis of gastroparesis should be made in the context of all available evaluable information.

Examples of patient reports that may be obtained by GEBT are as follows:

- Figure 4: Normal gastric emptying
- Figure 5: Delayed gastric emptying
- Figure 6: Borderline – conflicting diagnostic status at different time points
- Figure 7: Delayed at time points prior to 180 minutes but rising through the normal zone at 180 and 240 minute time points.

Figure 4. Sample GEBT Report – Patient with Normal Emptying

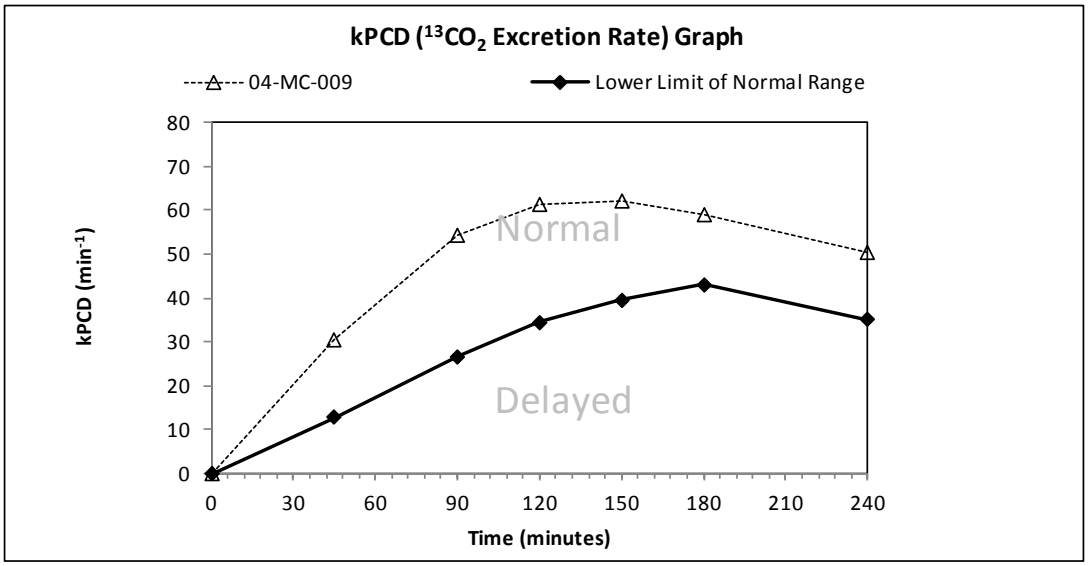
^{13}C-Spirulina Gastric Emptying Breath Test (GEBT) Patient Profile

Patient Name/ID	Test Date	Physician ID	Account #/ID	Report Date
04-MC-009	3-May-2006	Dr. Szarka	12345	7-May-2006

Status by kPCD			
Diagnostic Measurement	Results (min^{-1})	Normal Range (min^{-1})	Status
$kPCD_{45}$	30.4	≥ 12.9	**Normal**
$kPCD_{90}$	54.2	≥ 26.9	**Normal**
$kPCD_{120}$	61.2	≥ 34.4	**Normal**
$kPCD_{150}$	62.2	≥ 39.5	**Normal**
$kPCD_{180}$	58.9	≥ 43.0	**Normal**
$kPCD_{240}$	50.4	≥ 35.0	**Normal**

Definition and explanation of GEBT Metric "kPCD": kPCD is a mathematical expression of a test subject's $^{13}CO_2$ excretion rate (per minute, denoted as min^{-1}) at any measurement time *t*. kPCD is proportional to the rate of gastric emptying at time *t*; the higher the kPCD value the faster the rate of gastric emptying at that time point.

kPCD = 1000 x [%^{13}C Dose (in Test Meal) Excreted (as $^{13}CO_2$) per minute]



kPCD Excretion Curves (kPCD vs. time): Subjects with normal rates of gastric emptying typically display kPCD values that rise into the 120- to 180-minute time range and then decline thereafter. In contrast, kPCD values of delayed patients are lower and generally rise continuously throughout the 4-hour evaluation period.

Lab Director Approval _____ Date _____

Figure 5. Sample GEBT Report – Patient with Delayed Emptying

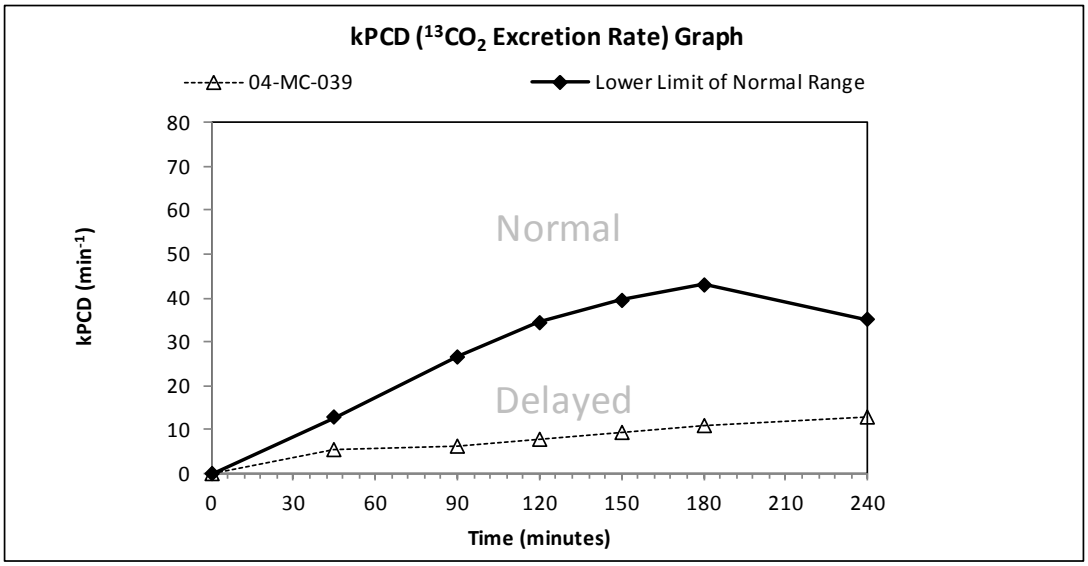
^{13}C-Spirulina Gastric Emptying Breath Test (GEBT) Patient Profile

Patient Name/ID	Test Date	Physician ID	Account #/ID	Report Date
04-MC-039	24-Jul-2006	Dr. Smith	12345	3-Dec-2013

Status by kPCD			
Diagnostic Measurement	Results (min^{-1})	Normal Range (min^{-1})	Status
$kPCD_{45}$	5.3	≥ 12.9	**Delayed**
$kPCD_{90}$	6.3	≥ 26.9	**Delayed**
$kPCD_{120}$	7.9	≥ 34.4	**Delayed**
$kPCD_{150}$	9.6	≥ 39.5	**Delayed**
$kPCD_{180}$	10.9	≥ 43.0	**Delayed**
$kPCD_{240}$	12.8	≥ 35.0	**Delayed**

Definition and explanation of GEBT Metric "kPCD": kPCD is a mathematical expression of a test subject's $^{13}CO_2$ excretion rate (per minute, denoted as min^{-1}) at any measurement time *t*. kPCD is proportional to the rate of gastric emptying at time *t*; the higher the kPCD value the faster the rate of gastric emptying at that time point.

kPCD = 1000 x [%^{13}C Dose (in Test Meal) Excreted (as $^{13}CO_2$) per minute]



kPCD Excretion Curves (kPCD vs. time): Subjects with normal rates of gastric emptying typically display kPCD values that rise into the 120- to 180-minute time range and then decline thereafter. In contrast, kPCD values of delayed patients are lower and generally rise continuously throughout the 4-hour evaluation period.

Lab Director Approval _____ Date _____

Figure 6. Sample GEBT Report – Patient with Borderline GEBT results

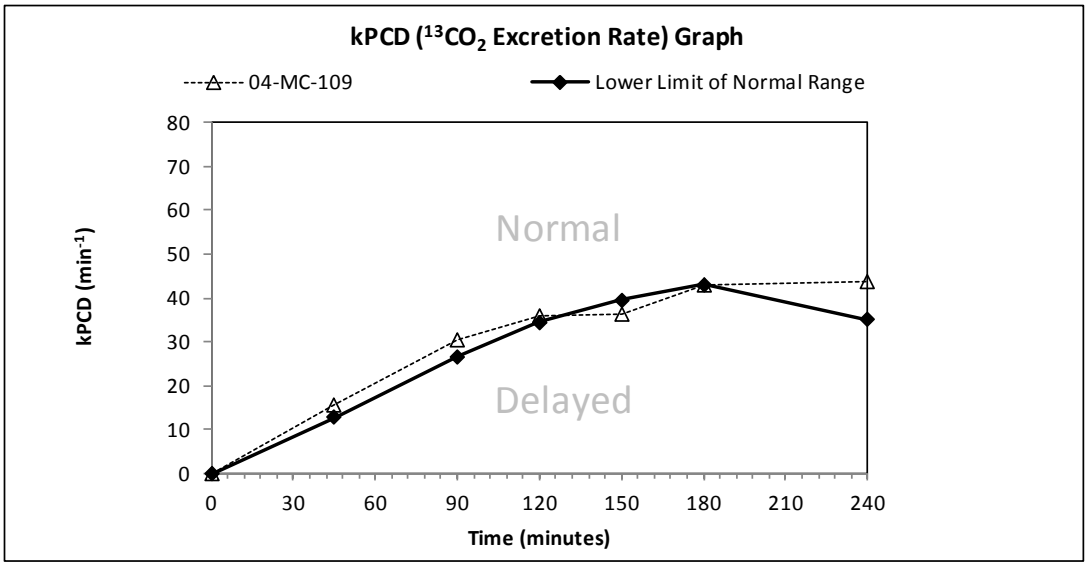
^{13}C-Spirulina Gastric Emptying Breath Test (GEBT) Patient Profile

Patient Name/ID	Test Date	Physician ID	Account #/ID	Report Date
04-MC-109	21-Dec-2006	Dr. Smith	12345	3-Dec-2013

Status by kPCD			
Diagnostic Measurement	Results (min^{-1})	Normal Range (min^{-1})	Status
$kPCD_{45}$	15.7	≥ 12.9	**Normal**
$kPCD_{90}$	30.5	≥ 26.9	**Normal**
$kPCD_{120}$	35.8	≥ 34.4	**Normal**
$kPCD_{150}$	36.2	≥ 39.5	**Delayed**
$kPCD_{180}$	43.1	≥ 43.0	**Normal**
$kPCD_{240}$	43.6	≥ 35.0	**Normal**

Definition and explanation of GEBT Metric "kPCD": kPCD is a mathematical expression of a test subject's $^{13}CO_2$ excretion rate (per minute, denoted as min^{-1}) at any measurement time *t*. kPCD is proportional to the rate of gastric emptying at time *t*; the higher the kPCD value the faster the rate of gastric emptying at that time point.

kPCD = 1000 x [%^{13}C Dose (in Test Meal) Excreted (as $^{13}CO_2$) per minute]



kPCD Excretion Curves (kPCD vs. time): Subjects with normal rates of gastric emptying typically display kPCD values that rise into the 120- to 180-minute time range and then decline thereafter. In contrast, kPCD values of delayed patients are lower and generally rise continuously throughout the 4-hour evaluation period.

Lab Director Approval _____ Date _____

Figure 7. Sample GEBT Report – Patient with Predominantly Delayed GEBT results

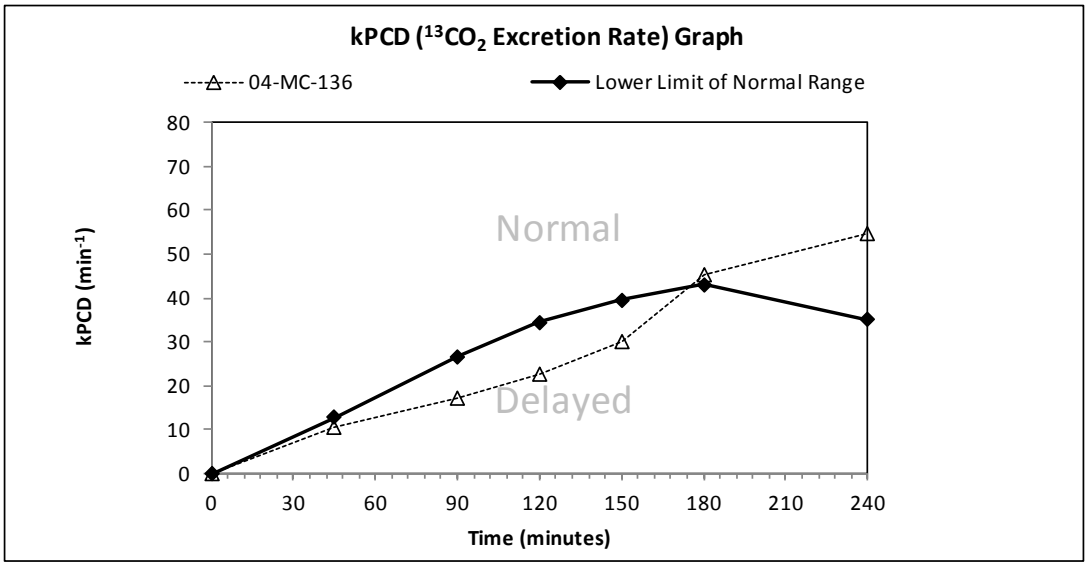
^{13}C-Spirulina Gastric Emptying Breath Test (GEBT) Patient Profile

Patient Name/ID	Test Date	Physician ID	Account #/ID	Report Date
04-MC-136	26-Feb-2007	Dr. Smith	12345	3-Dec-2013

Status by kPCD			
Diagnostic Measurement	Results (min^{-1})	Normal Range (min^{-1})	Status
$kPCD_{45}$	10.7	≥ 12.9	**Delayed**
$kPCD_{90}$	17.1	≥ 26.9	**Delayed**
$kPCD_{120}$	22.7	≥ 34.4	**Delayed**
$kPCD_{150}$	30.0	≥ 39.5	**Delayed**
$kPCD_{180}$	45.1	≥ 43.0	**Normal**
$kPCD_{240}$	54.9	≥ 35.0	**Normal**

Definition and explanation of GEBT Metric "kPCD": kPCD is a mathematical expression of a test subject's $^{13}CO_2$ excretion rate (per minute, denoted as min^{-1}) at any measurement time *t*. kPCD is proportional to the rate of gastric emptying at time *t*; the higher the kPCD value the faster the rate of gastric emptying at that time point.

kPCD = 1000 x [%^{13}C Dose (in Test Meal) Excreted (as $^{13}CO_2$) per minute]



kPCD Excretion Curves (kPCD vs. time): Subjects with normal rates of gastric emptying typically display kPCD values that rise into the 120- to 180-minute time range and then decline thereafter. In contrast, kPCD values of delayed patients are lower and generally rise continuously throughout the 4-hour evaluation period.

Lab Director Approval _____ Date _____

12. Validation of GEBT versus Scintigraphy

12.1 Validation Overview

The GEBT was validated against the reference method of gastric scintigraphy. The GEBT validation plan utilized an ordered sequence of four studies; the first three studies were pre-validation studies which determined the test meal matrix and configuration of the GEBT, measured biologic variability and established normal Reference Ranges for GEBT and the gold standard method of gastric scintigraphy. The fourth study was the pivotal validation study.

12.2 Validation Study Design

This study determined the diagnostic concordance (overall diagnostic agreement), sensitivity and specificity of GEBT compared to gastric scintigraphy for the diagnosis of delayed gastric emptying (gastroparesis).

The study was a prospective, open-label, method comparison validation study. Enrollment was designed to continue until up to 50, but not less than 45, scintigraphically positive (gastroparetic) individuals had been tested and an overall total of 115 evaluable individuals were tested. A total of 132 subjects were enrolled and 129 of those completed the study. Enrollment is summarized as follows:

1. One-hundred and two (102) subjects were recruited from patients suspected of gastroparesis and scheduled for gastric scintigraphy.
2. Twenty-four (24) subjects were enrolled who had been previously suspected of and evaluated for gastroparesis.
3. To supplement the number of scintigraphically positive subjects, five healthy individuals were enrolled for pharmacological induction of delayed gastric emptying by intravenous injection of atropine.
4. One (1) of 11 diabetic subjects enrolled in the study was an asymptomatic patient from the Diabetology Clinic enrolled upon recommendation of the attending physician.

To eliminate the effects of natural, day-to-day variation in gastric emptying on comparative diagnostic results from scintigraphy and GEBT in this validation study, each individual was <u>simultaneously</u> tested by scintigraphy and GEBT using a dual-label test meal. The term dual-label refers to a test meal that contains both the GEBT labeling material (^{13}C-Spirulina) and the labeling substance used in scintigraphy (^{99m}Tc Sulphur colloid). After consuming the standardized GEBT meal containing both labels, gastric emptying testing of each individual was conducted simultaneously by scintigraphy and GEBT at 45, 90, 120, 150, 180 and 240 minute post-meal measurement times.

Of the 129 study subjects who completed the study, protocol deviations affected results for 14 subjects, yielding 115 evaluable subjects. Performance statistics are based on the 115 evaluable subjects.

12.3 Validation Study Results

12.3.1 Performance Characteristics: Diagnosis of Delayed Gastric Emptying

The diagnostic status (delayed vs. normal) of each subject was determined at each measurement time by GEBT and by scintigraphy, using the GEBT and scintigraphic cut-off points (COPs) established prior to conducting the validation study. Diagnostic classification by GEBT was then compared to diagnostic classification by scintigraphy.

In the validation study, the prevalence (positivity rate) of delayed gastric emptying as determined by scintigraphy varied from 22% at 45 minutes to a maximum of ~50% at 120 minute. Based on diagnostic classification of each patient by each method, GEBT performance characteristics (Sensitivity, Specificity, Concordance, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) vs. Scintigraphy are reported in Table 5.

Performance statistics by GEBT metric are tabulated below. For simplicity, the acronym for the metric "Scintigraphic Fraction of Test Meal Emptied" is Scint_FE.

Table 5. Diagnosis by GEBT vs. Scintigraphy

	Diagnosis by kPCD vs. Scint_FE at 45, 90, 120, 150, 180 and 240 minutes from the end of test meal consumption					
Classification (N = 115)	**45**	**90**	**120**	**150**	**180**	**240**
TP*	16	30	31	29	20	11
TN†	84	63	57	60	57	67
FP‡	6	2	1	2	7	2
FN§	9	20	26	24	31	35
Performance Statistics (%)						
Specificity (95% CI)	93.3 (88.1-98.5)	96.9 (92.8-100)	98.3 (94.9-100)	96.8 (96.8-100)	89.1 (81.4-96.6)	97.1 (93.3-100)
Sensitivity (95% CI**)	64.0 (42.2-83.0)	60.0 (45.2-72.9)	54.4 (40.7-65.7)	54.7 (40.2-67.5)	39.2 (27.1-53.3)	23.9 (11.6-37.6)
Concordance	87.0	80.9	76.5	77.4	67.0	67.8
PPV¶ (95% CI)	72.7 (55.5-89.6)	93.8 (88.4-100)	96.9 (90.9-100)	93.5 (85.3-100)	74.1 (59.5-89.8)	84.6 (66.3-100)
NPV†† (95% CI)	90.3 (85.1-95.0)	75.9 (63.7-78.0)	68.7 (62.4-74.1)	71.4 (65.0-77.3)	64.8 (59.7-70.8)	65.7 (61.9-70.0)

* TP: true positive; † TN: true negative; ‡ FP: false positive; § FN: false negative; ¶ PPV: positive predictive value; **CI: confidence interval, bootstrap; †† NPV: negative predictive value

Estimated values of Positive Predictive Value (PPV) and Negative Predictive Value (NPV) vs. Scintigraphy over the range of prevalence rates of gastroparesis reported in the literature from 27% to 57% are also reported (Table 6).

Table 6. Performance Statistics (PPV and NPV) of GEBT vs. Scintigraphy Adjusted for Differing Rates of Prevalence (27% and 57%)

	Diagnosis by kPCD vs. Scint_FE at 45, 90, 120, 150, 180 and 240 minutes from the end of test meal consumption					
Time Point	**45**	**90**	**120**	**150**	**180**	**240**
PPV 27% Prevalence (95% CI)	78.0 (62.9-92.5)	87.8 (73.5-100)	92.1 (78.6-100)	85.3 (70.7-100)	57.0 (38.7-78.2)	75.3 (50.4-100)
NPV 27% Prevalence (95% CI)	87.5 (81.0-93.4)	86.8 (82.5-90.5)	85.4 (81.6-88.5)	86.3 (81.2-88.8)	79.8 (76.2-84.0)	77.5 (74.6-80.8)
PPV 57% Prevalence (95% CI)	92.7 (86.4-98.4)	96.3 (91.2-100)	97.7 (93.1-100)	95.7 (90.1-100)	82.6 (71.5-94.2)	91.6 (80.0-100)
NPV 57% Prevalence (95% CI)	66.2 (53.1-78.7)	64.6 (56.4-72.3)	61.9 (55.0-68.0)	61.7 (54.3-68.6)	52.5 (46.9-59.1)	49.1 (44.9-53.9)

12.4 Validation Study Limitations

12.4.1 Tobacco Use

A conclusive effect of tobacco use/smoking upon rates of gastric emptying could not be assessed from the validation study, thus results from current smokers should be interpreted with caution.

12.4.2 *H. pylori*

A conclusive effect of *H. pylori* on GEBT results was not established from the validation study, thus results from subjects that tested positive for *H. pylori* should be interpreted with caution

12.4.3 Diagnostic performance statistics

Tables 5 and 6 (Section 12.3.1) demonstrate the performance statistics of GEBT as compared to scintigraphy from the validation study and results do not reflect the diagnostic performance of GEBT as compared to clinical diagnosis of delayed gastric emptying.

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