

Figure 2. Discrimination between Normal, Delayed and Borderline Subjects by ¹³C-Spirulina GEBT

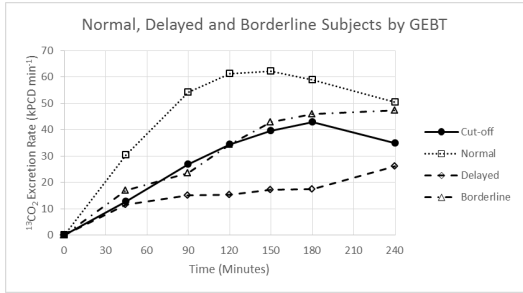
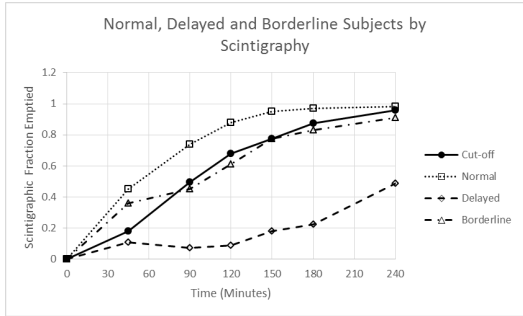


Figure 3. Discrimination between Normal, Delayed and Borderline Subjects by Scintigraphy



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 ¹³CO₂ 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 ¹³C-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 ¹³C-Spirulina GEBT may not be consistent across all time points for some patients. ¹³C-Spirulina GEBT results are intended to provide supplemental information to the clinician when evaluating a patient for gastroparesis. ¹³C-Spirulina 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 ¹³C-Spirulina 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.

12. Validation of ¹³C-Spirulina GEBT versus Scintigraphy

12.1 Validation Overview

The ¹³C-Spirulina GEBT was validated against the reference method of gastric scintigraphy. The ¹³C-Spirulina 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 ¹³C-Spirulina GEBT, measured biologic variability and established normal Reference Ranges for ¹³C-Spirulina GEBT and the gold standard method of gastric scintigraphy. The fourth study was the pivotal validation study.

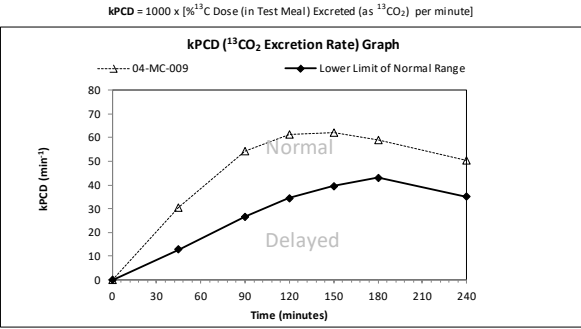
12.2 Validation Study Design

This study determined the diagnostic concordance (overall diagnostic agreement), sensitivity and specificity of ¹³C-Spirulina GEBT compared to gastric scintigraphy for the diagnosis of delayed gastric emptying (gastroparesis).

Figure 4. Sample ¹³C-Spirulina 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 ¹³CO₂ 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.



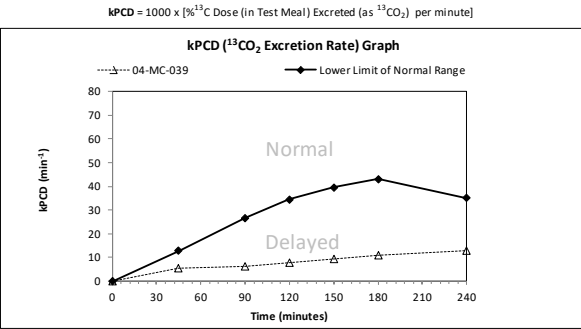
kPCD Excretion Curves (kPCD vs. time): Subjects with normal rates of gastric emptying typically display kPCD values that rise into the 120- to 180-minute time range and then decline thereafter. In contrast, kPCD values of delayed patients are lower and generally rise continuously throughout the 4-hour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

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Figure 5. Sample ¹³C-Spirulina 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 ¹³CO₂ 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.



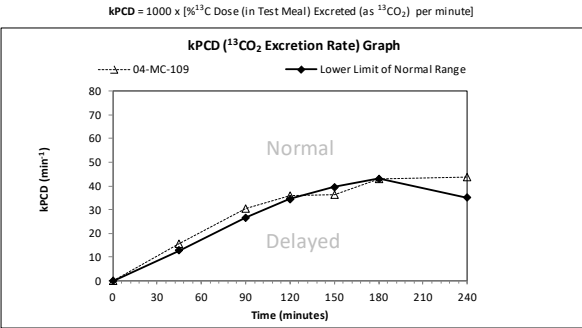
kPCD Excretion Curves (kPCD vs. time): Subjects with normal rates of gastric emptying typically display kPCD values that rise into the 120- to 180-minute time range and then decline thereafter. In contrast, kPCD values of delayed patients are lower and generally rise continuously throughout the 4-hour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

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Figure 6. Sample ¹³C-Spirulina GEBT Report – Patient with Borderline ¹³C-Spirulina 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 ¹³CO₂ 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.



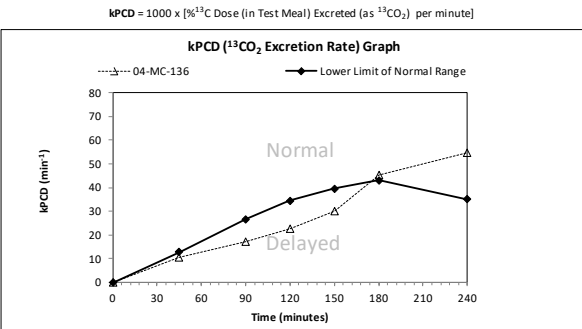
kPCD Excretion Curves (kPCD vs. time): Subjects with normal rates of gastric emptying typically display kPCD values that rise into the 120- to 180-minute time range and then decline thereafter. In contrast, kPCD values of delayed patients are lower and generally rise continuously throughout the 4-hour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

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Figure 7. Sample ¹³C-Spirulina GEBT Report – Patient with Predominantly Delayed ¹³C-Spirulina 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 ¹³CO₂ 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 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.

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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 ¹³C-Spirulina GEBT in this validation study, each individual was simultaneously tested by scintigraphy and ¹³C-Spirulina GEBT using a dual-label test meal. The term dual-label refers to a test meal that contains both the ¹³C-Spirulina GEBT labeling material (¹³C-Spirulina) and the labeling substance used in scintigraphy (^{99m}Tc Sulphur colloid). After consuming the standardized ¹³C-Spirulina GEBT meal containing both labels, gastric emptying testing of each individual was conducted simultaneously by scintigraphy and ¹³C-Spirulina 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 ¹³C-Spirulina GEBT and by scintigraphy, using the ¹³C-Spirulina GEBT and scintigraphic cut-off points (COPs) established prior to conducting the validation study. Diagnostic classification by ¹³C-Spirulina 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 minutes. Based on diagnostic classification of each patient by each method, ¹³C-Spirulina 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 ¹³C-Spirulina 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 ¹³ C-Spirulina 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	93.3	96.9	98.3	96.8	89.1	97.1
(95% CI)	(88.1-98.5)	(92.8-100)	(94.9-100)	(96.8-100)	(81.4-96.6)	(93.3-100)
Sensitivity	64.0	60.0	54.4	54.7	39.2	23.9
(95% CI**)	(42.2-83.0)	(45.2-72.9)	(40.7-65.7)	(40.2-67.5)	(27.1-53.3)	(11.6-37.6)
Concordance	87.0	80.9	76.5	77.4	67.0	67.8
PPV†	72.7	93.8	96.9	93.5	74.1	84.6
(95% CI)	(55.5-89.6)	(88.4-100)	(90.9-100)	(85.3-100)	(59.5-89.8)	(66.3-100)
NPV††	90.3	75.9	68.7	71.4	64.8	65.7
(95% CI)	(85.1-95.0)	(63.7-78.0)	(62.4-74.1)	(65.0-77.3)	(59.7-70.8)	(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 ¹³ C-Spirulina 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	78.0	87.8	92.1	85.3	57.0	75.3
(95% CI)	(62.9-92.5)	(73.5-100)	(78.6-100)	(70.7-100)	(38.7-78.2)	(50.4-100)
NPV 27% Prevalence	87.5	86.8	85.4	86.3	79.8	77.5
(95% CI)	(81.0-93.4)	(82.5-90.5)	(81.6-88.5)	(81.2-88.8)	(76.2-84.0)	(74.6-80.8)
PPV 57% Prevalence	92.7	96.3	97.7	95.7	82.6	91.6
(95% CI)	(86.4-98.4)	(91.2-100)	(93.1-100)	(90.1-100)	(71.5-94.2)	(80.0-100)
NPV 57% Prevalence	66.2	64.6	61.9	61.7	52.5	49.1
(95% CI)	(53.1-78.7)	(56.4-72.3)	(55.0-68.0)	(54.3-68.6)	(46.9-59.1)	(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 ¹³C-Spirulina 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 ¹³C-Spirulina GEBT as compared to scintigraphy from the validation study and results do not reflect the diagnostic performance of ¹³C-Spirulina GEBT as compared to clinical diagnosis of delayed gastric emptying.

13. Bibliography

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14. Name and Place of Business

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15. Date of Issuance

January 2026

L075R04