Effective Date: OCT 14 2020

Title Page

Protocol Title: ABCA2 GIRMS Analytical Validation Clinical Performance Study – Patient Sample Collection

Protocol Number: PRO-CD-044

Amendment Number: 01

Sponsor Name: Advanced Breath Diagnostics, LLC, dba Cairn Diagnostics

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1.0 STUDY SYNOPSIS

The purpose of this study is to collect human breath samples for use in a validation study intended to demonstrate equivalent clinical performance measures of new ABCA2 GIRMS instruments to the currently FDA-approved ABCA GIRMS system. ABCA-GIRMS systems are used to analyze the ratio of $^{13}CO_2$ to $^{12}CO_2$ in patient breath samples collected during the GEBT test procedure.

In this study the participants will be administered the FDA-approved GEBT test, which involves collecting breath samples prior to and after consumption of a non-radioactive, carbon stable isotope (^{13}C) labeled test meal. Breath samples will be collected at times consistent with FDA approved labeling. Participant's breath samples will be analyzed on the currently approved ABCA GIRMS and on new ABCA2 GIRMS instruments. Resulting DOB values (delta over baseline: difference between pre-meal and post-meal $^{13}CO_2$ to $^{12}CO_2$ ratios) and kPCD values ($^{13}CO_2$ excretion rate (min^{-1}) primary GEBT metric) will be recorded. The DOB and kPCD values will be used to determine the clinical diagnostic agreement at each individual time points and percent agreement in diagnosis between the approved ABCA GIRMS and the new ABCA2 GIRMS.

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 ("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.

Term/Abbreviation	Definition
DOB	Delta Over Baseline i.e. the difference in delta value at any given time point and the pre-meal/baseline delta value. Used in kPCD calculations.
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
kPCD	1000 x Percent Dose (of Carbon-13) Excreted, $^{13}CO_2$ excretion rate (min^{-1}) primary GEBT metric
PHI	Personal Health Information
SAE	Serious Adverse Event
WOCBP	Woman 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.

Note: The addition of the new ABCA2 GIRMS system as a component of ^{13}C-Spirulina GEBT will not change the intended use of GEBT. The new ABCA2 GIRMS instruments are intended to serve the same analytical function and use the same analytical platform and analytical methodology as the original ABCA-GIRMS to determine the ratio of $^{13}CO_2$ to $^{12}CO_2$ in human breath samples.

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
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

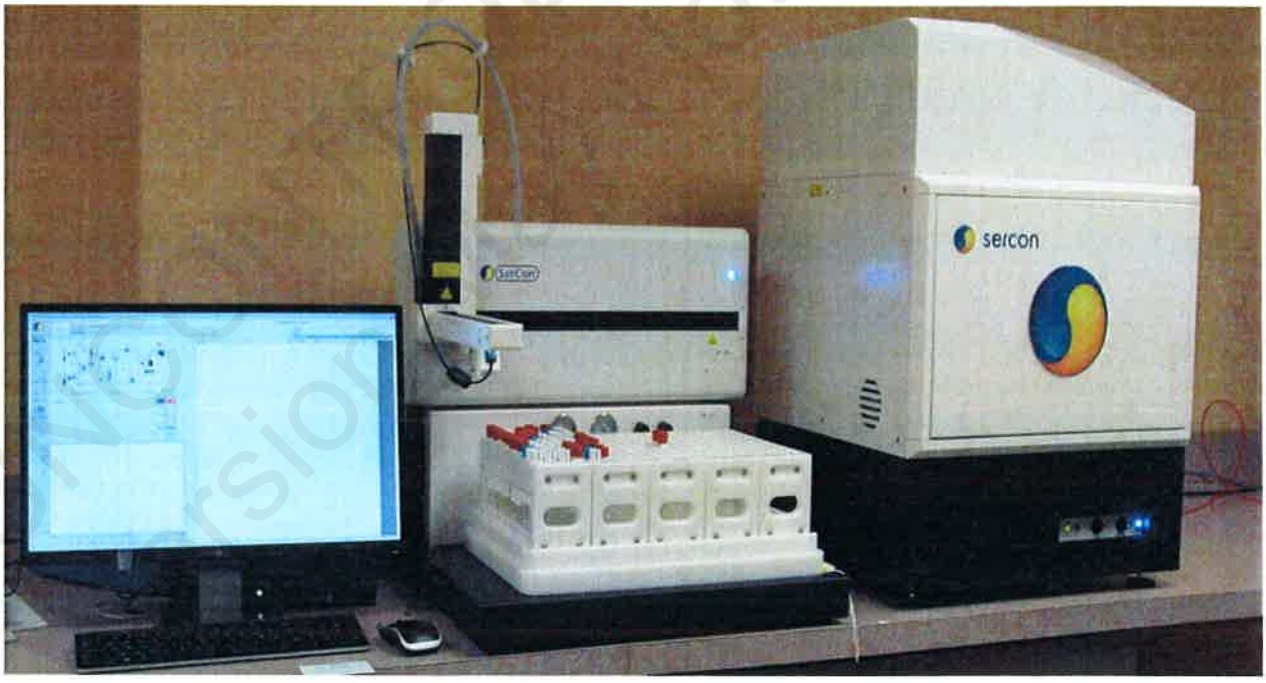

Figure 4. New ABCA2 GIRMS instrument

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 below 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-039, ABCA2 GIRMS Analytical Validation Precision Study – Patient Sample Collection, was designed to collect human breath samples for use in a validation study intended to demonstrate equivalent analytical precision of new ABCA2 GIRMS instruments to the FDA-approved ABCA GIRMS system. In this study, the participant's breath samples were analyzed on the FDA-approved ABCA GIRMS and on the new ABCA2 GIRMS instruments. The resulting delta ($\delta^{13}C$) values ($^{13}CO_2$ to $^{12}CO_2$ ratios) and DOB values (delta over baseline: difference between pre-meal and post-meal $^{13}CO_2$ to $^{12}CO_2$ ratios) were recorded and used to calculate within and between instrument precision performance characteristics for the ABCA GIRMS systems.

PRO-CD-042, ABCA2 GIRMS Analytical Validation Interference/Specificity Study – Patient Sample Collection was designed to collect human breath samples for use in a validation study intended to demonstrate comparable performance characteristics of new ABCA2 GIRMS instruments to the FDA-approved ABCA GIRMS system. In this study, the participants had breath samples collected prior to and after administration of a potential interfering substance that were identified in the original validation of the ABCA GIRMS system or identified as possibly present in breath samples in the ABCA2 GIRMS risk assessment. The samples were analyzed on either the ABCA2 GIRMS (all identified potential interferences) or the FDA approved ABCA GIRMS (newly identified potential interferences). The resulting delta ($\delta^{13}C$) values ($^{13}CO_2$ to $^{12}CO_2$ ratios) were recorded and used to determine the interference/specificity of the ABCA GIRMS systems.

4.0 STUDY GOALS AND OBJECTIVES

The purpose of this study is to collect human breath samples for use in a validation study intended to demonstrate equivalent clinical performance of new ABCA2 GIRMS instruments to the current FDA-approved ABCA GIRMS system. The clinical performance data will be used as part of a supplement submission to FDA demonstrating that both ABCA2 GIRMS systems are suitable for use with GEBT and that the safety and efficacy of GEBT is not affected.

5.0 SUBJECT SELECTION

Cairn personnel and their family and friends may volunteer to be a participant in this study.

Participants may also be recruited from clinical trial sites that have a population that is known to have a history of possible gastric emptying issues (intended use population for GEBT).

This study requires a minimum of seventy-five (75) and up to one hundred and twenty (120) 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 from healthy and intended use population participants (i.e. symptomology of gastroparesis). Women of childbearing potential (WOCBP) must not be pregnant at the time of GEBT administration.
- Ability to eat test meal and provide breath samples

5.2 Exclusion criteria

- History or physical exam suggestive of pathophysiologic disorders such as renal failure, chronic heart disease, chronic respiratory disease, liver disease, or malabsorption syndrome
- 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 at a GEBT trial site 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-035, 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 GEBT test site at which time the GEBT will be administered. After confirmation that the participant has met the fasting and dietary restriction requirements, the participant will provide a baseline (PRE-meal) breath sample by exhaling into two blue-capped glass Exetainer tubes. The participant will then consume the prepared GEBT test meal. After consuming the test meal, post meal breath samples will likewise be collected at 45, 90, 120, 150, 180 and 240-minute timepoints.

Visit 3: 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	Follow-up (Visit 3)	Notes
Informed Consent	X			
Inclusion and Exclusion Criteria	X			
Administration of ^{13}C-Spirulina GEBT		X		
AE review			X	
SAE review			X	

6.2 Handling and analysis of breath samples

The handling and analysis of breath samples collected by this protocol is fully described in PRO-QC-202, ABCA2 GIRMS Clinical Performance Measures (Exhibit B). In summary, human breath samples collected will be analyzed on the original ABCA GIRMS system. The samples will then be analyzed on either the ABCA-C or ABCA-D ABCA2 GIRMS systems and then analyzed a third time on the ABCA2 GIRMS system on which the breath samples have not been analyzed.

6.3 End of Study

The participant is considered to have completed the study when they have completed the GEBT test administration and all collected breath samples have been analyzed on the approved ABCA GIRMS and two new ABCA2 GIRMS systems.

The end of the study is defined as the date on which the analysis of all the participant's breath samples have been completed on the GIRMS systems.

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 with 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 with 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.

8.6 Pregnancy

If a pregnancy is reported, the investigator should inform the sponsor within 24 hours of learning of the pregnancy and should follow the procedures outlined in Exhibit F.

Abnormal pregnancy outcomes (e.g. spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

9.0 STUDY OVERSIGHT

Quality and compliance monitoring will include periodic visits by Cairn Diagnostics representative(s) of sufficient frequency to assure

- The study protocol is being followed as approved by Cairn Diagnostics and the IRB;
- Informed consent is being obtained before any procedures are conducted;
- Accurate, complete and current records are being maintained in a timely manner;
- Accurate, complete and current reports are being made to Cairn Diagnostics and the IRB; and
- The investigator has adequate resources to complete the study and has not delegated responsibilities to unspecified or untrained personnel.

10.0 PRODUCT STORAGE AND ACCOUNTABILITY

GEBT kits will be distributed to the investigator, or designee as needed for the course of the study.

Only the participants enrolled in the study may receive the GEBT kits and only authorized personnel may supply or administer the GEBT. All GEBT kits must be 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 prior to shipping samples to Cairn Diagnostics (Brentwood, TN USA) for analysis.

All components of an unused GEBT kit may be disposed of into a trash receptable.

The investigator or institution is responsible for GEBT kit accountability, reconciliation, and record maintenance (i.e. receipt, reconciliation, and final disposition records).

11.0 MEASURES TO MINIMIZE BIAS: RANDOMIZATION

The ABCA GIRMS (A) system utilizes a larger sample of breath (~4mL) than the new ABCA2 GIRMS (C and D) systems (~1-2 mL). For this reason, all samples will be analyzed on ABCA-A first. In order to minimize any bias caused by running the same samples on

different instruments in the same order, the order in which samples are analyzed will be randomized as follows:

	Study Participant Breath Sample Analysis Order		
Subject Number	ABCA GIRMS (A)	ABCA2 GIRMS (C)	ABCA2 GIRMS (D)
001	1	2	3
002	1	3	2
003	1	3	2
004	1	2	3
Etc	Etc	Etc	Etc

12.0 DATA MANAGEMENT

12.1 Breath Sample Analysis

GEBT breath samples will be shipped to Cairn Diagnostics clinical laboratory in Brentwood, Tennessee USA for analysis using the original ABCA GIRMS and new ABCA2 GIRMS systems according to each systems Standard Operating Procedure (SOP-QC-017, Operation, Calibration and Maintenance – ABCA GIRMS and SOP-QC-090, Operation, Calibration and Maintenance – ABCA2 GIRMS). Analysis will be conducted by trained and qualified clinical laboratory personnel with verified and documented training appropriate for operation of ABCA GIRMS systems.

12.2 ^{13}C-Spirulina GEBT reporting

Generate GEBT reports according to Cairn procedure SOP-CD-005, Breath Test Processing, for study participant breath samples analyzed on the ABCA GIRMS and ABCA2 GIRMS systems to determine the diagnostic agreement and at each individual timepoint and the percent agreement in diagnosis between the ABCA GIRMS and the two new ABCA2 GIRMS.

All data generated from each individual report will be entered into a spreadsheet for statistical analysis.

Participants may be provided with their GEBT results from the breath samples analyzed on the FDA-approved ABCA GRIMS system. Results generated from the ABCA2 GIRMS will not be provided to participants as the validation study of these instruments is still ongoing.

12.3 Sample retention

Cairn Diagnostics clinical laboratory will 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

Perform statistical analysis according to:

1. Assay Migration Studies for In Vitro Diagnostic Devices – Guidance for Industry and FDA Staff
2. CLSI Guidance EP12-A2, User Protocol for Evaluation of Qualitative Test Performance; Approved Guidance – Second Edition
3. CLSI Guidance EP21, Evaluation of Total Analytical Error for Quantitative Medical Laboratory Measurement Procedures; Second Edition

Specific formulas and calculations are found in Exhibits B and C, PRO-QC-202, ABCA2 GIRMS Clinical Performance Measures and PRO-QC-191, ABCA2 GIRMS Analytical Validation – Bias and Total Analytical Error.

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.

Summarize the results obtained from the execution of the precision protocol in the report for PRO-QC-202, ABCA2 GIRMS Clinical Performance Measures.

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-035) must be provided to the participant.

13.3 Financial Disclosure

Investigators and sub-investigators will provide Cairn Diagnostics with sufficient, accurate financial information as requested to allow the sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities.

Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after the completion of the study.

13.4 Data Protections

Assign each participant a unique identifier. Since Cairn Diagnostics is the study investigator as well as the sponsor, Cairn, its principal investigator, and study team will need to receive and use certain personal information of the study subjects. If Cairn needs to share a subject's study related data with an individual or entity outside of Cairn, other than the subject's study doctor or his/her study staff, it will replace any information which could identify the subject with a unique code number. Only study team members will have access to the key that links the assigned code to the study subject.

Inform the participant that his/her personal study-related data will be used by Cairn Diagnostics in accordance with local data protection law.

Inform the participant that his/her medical records may be examined Cairn Diagnostics auditors, other authorized personnel appointed by Cairn Diagnostics, by appropriate IRB/IEC member and by inspectors from regulatory authorities.

Obtain a HIPAA authorization to collect, use and disclose the study subject's personal information and protected health information as needed to conduct the study (refer to SPEC-CD-035).

13.5 Data Quality Assurance

All participant data relating to the study will be recorded on printed case report forms (CRFs). The investigator is responsible for verifying that data entries are accurate and correct by signing the CRF.

The investigator must maintain accurate documentation (source data) that supports the information entered in the CRF. Source documents provide evidence for the existence of the participant and substantiate the integrity of the data collected.

The investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspectors and provide direct access to source data documents.

Cairn Diagnostics is responsible for the data management of this study including quality data checking of the data.

Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source

documents; the safety and rights of the participants are being protected and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

Records and documents, including signed informed consent forms, pertaining to the conduct of this study must be retained by the investigator for 2 years after study completion unless local regulations or institutional policies require a longer retention period. No records may be destroyed during the retention period without the written approval of Cairn Diagnostics. No records may be transferred to another location or party without written notification to Cairn.

13.6 Study and Site Closure

Cairn Diagnostics reserves the right to close the study site or terminate the study at any time for any reason. Study sites will be closed upon study completion. A study site is considered closed when all required and study supplies have been collected and a study-site closure visit has been performed.

The investigator may initiate study site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study by the sponsor or investigator may include but not limited to:

- Failure of the investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the sponsor's procedures or GCP guideline
- Inadequate recruitment of participants by the investigator
- Discontinuation of further study intervention development

14.0 INTENDED USE OF DATA

Breath samples obtained in this study will be used to confirm an acceptable level of clinical performance measures of the ABCA2 GIRMS systems and demonstrate comparable performance characteristics to the original ABCA GIRMS system and will be submitted to FDA as a supplement to PMA P110015.

15.0 REFERENCES

A. EP12-A2, User Protocol for Evaluation of Qualitative Test Performance; Approved Guidance – Second Edition
B. EP21, Evaluation of Total Analytical Error for Quantitative Medical Laboratory Measurement Procedures; Second Edition
C. Assay Migration Studies for In Vitro Diagnostic Devices – Guidance for Industry and FDA Staff.
D. 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
E. 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

F. 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.
G. PRO-QC-191, ABCA2 GIRMS Analytical Validation – Bias and Total Analytical Error
H. PRO-QC-202, ABCA2 GIRMS Clinical Performance Measures
I. SOP-CD-005, Breath Test Processing
J. SOP-QC-017, Operation, Calibration and Maintenance – ABCA GIRMS
K. SOP-QC-030, Data Generation and Review
L. SOP-QC-090, Operation, Calibration and Maintenance – ABCA2 GIRMS
M. SPEC-CD-035, Informed Consent Form – Study PRO-CD-044
N. SPEC-QA-291, ^{13}C-Spirulina GEBT Administration Instructions

16.0 EXHIBITS

A. Exhibit A P110015 Approval and FDA Press Release
B. Exhibit B PRO-QC-202, ABCA2 GIRMS Clinical Performance Measures
C. Exhibit C PRO-QC-191, ABCA2 GIRMS Analytical Validation – Bias and Total Analytical Error
D. Exhibit D COVID-19 Transmission Mitigation Plan
E. Exhibit E Study Participant Heath Questionnaire
F. Exhibit F Contraceptive Guidance and Collection of Pregnancy Information

17.0 REVISION HISTORY

Revision Level	Brief Description of Revision(s)	Effective Date
00	Initial document	9/3/20
01	Updated Section 5.1, Inclusion Criteria and Section 6.1 Study Scheme to remove the requirement of performing a pregnancy test prior to administration of GEBT; Updated Section 13.4, Data Protections, to clarify Cairn procedures for study participant personal information data protections and requirement for obtaining HIPAA authorization from the study participant to disclose the relevant information to Cairn that is needed to conduct the study; Added section 16.0 Exhibits; Corrected Exhibit D, COVID-19 Transmission Mitigation Plan to remove reference to collecting samples in breath bags; Clarified that the COVID-19 Transmission Mitigation Plan is the plan performed by Cairn personnel and the sub-investigators will perform their own COVID-19 mitigation procedures	See first page of this document.

Principal Investigator Agreement

In accordance with Good Clinical Practice (GCP) and Food and Drug Administration (FDA) regulations, 21 CFR Part 812, Investigational Device Exemptions (IDE), and 21 CFR Part 50, Protection of Human Subjects, this document serves as an Investigator Agreement for:

By signing this form, I commit to the following:

1. I will conduct the clinical investigation in accordance with this agreement, all requirements of the investigational plan, IDE regulations, other applicable regulations of the FDA, and any conditions of approval imposed by my reviewing Institutional Review Board (IRB) or FDA. I agree to abide by all of the responsibilities of investigators addressed under 21 CFR Part 812, Subpart E and Subpart G.
2. I have the appropriate, relevant qualifications to conduct and to oversee the conduct of the clinical trial. I have provided my most recent curriculum vitae (CV), which details my relevant qualifications, including dates, location, extent, and type of experience.
3. I certify there are no reasons to question my ability to oversee the appropriate conduct of this clinical trial.
    - I *have never* participated in a clinical trial or other research activity which was terminated (disqualified) by FDA, the IRB (or equivalent), or sponsor of a study due to a non-compliance issue;

    - OR -

    - I *have* participated in a clinical trial or other research activity which was terminated (disqualified) by FDA, the IRB (or equivalent), or sponsor of a study due to a non-compliance issue. I have provided documentation with further details describing the specific circumstances leading to this termination and my role in the respective problems or issues and the resolution of these problems or issues.
4. I certify that I have not been debarred under the Generic Drug Enforcement Act of 1992, 21 USC 335a and 335b. In the event that I become debarred or receive notice of an action or threat of an action with respect to my debarment during the term of this Agreement, I agree to immediately notify the sponsor/sponsor-investigator and the authorized IRB for my study site.
5. As required by 21 CFR Part 812, I have disclosed complete and accurate financial information to the applicable parties involved in this study. I will update my disclosed financial information with changes at any time during the clinical trial following the completion of the study, per applicable regulations.

Printed Name: Alex Ryder

Signature: [signature] **Date:** 10/13/26

18.0 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

19.0 EXHIBIT B. PRO-QC-202.00, ABCA2 GIRMS CLINICAL PERFORMANCE MEASURES

Effective Date: SEP 03 2020

1.0 PURPOSE

This protocol describes the comparison of the clinical performance of the original ABCA-GIRMS (Automated Breath Carbon Analyzer – Gas Isotope Ratio Mass Spectrometer) and new ABCA2-GIRMS systems when used to analyze ratios of $^{13}CO_2$ to $^{12}CO_2$ in breath samples collected using Cairn's ^{13}C-Spirulina GEBT, that are used to calculate $^{13}CO_2$ excretion rates (kPCD min^{-1}) that describe gastric emptying rates.

2.0 BACKGROUND

The performance characteristics of the ABCA GIRMS instrument currently in use for analyzing carbon stable isotope ratios of CO_2 in breath samples collected during administration of the GEBT were established and documented in PRO-QC-114.01, Analytical Performance of ABCA GIRMS Instruments, and the associated report (submitted as part of PMA P110015).

This protocol includes studies that seek to confirm the acceptable level of clinical performance measures of the ABCA2 GIRMS system and demonstrate comparable performance characteristics to the original ABCA GIRMS system. This study will be performed in accordance with CLSI Guidance EP12-A2, User Protocol or Evaluation of Qualitative Test Performance; Approved Guideline, Second Edition and Assay Migration Studies for In Vitro Diagnostic Devices – Guidance for Industry and FDA Staff.

3.0 RESPONSIBILITIES

Requirement	Responsibility
Protocol generation	Director of Quality Assurance and Regulatory Compliance
Protocol approval	Clinical Laboratory Director, Chief Information and Instrumentation Officer, Director of Laboratory Operations
Protocol execution	Clinical Laboratory personnel
Data analysis	Data Management Associate, Director of Laboratory Operations, Director of Quality Assurance and Regulatory Compliance, Chief Information and Instrumentation Officer
Report generation	Director of Quality Assurance and Regulatory Compliance

	NAME (Print)	SIGNATURE	DATE
Originator:	CATHERINE WILLIAMS		9-2-2020
Approved By:	Samantha Baudhuin		9/1/20
Approved By:	Shane Crabtree		9/2/2020
Approved By:	See	Attached cc	9-3-20

Effective Date:

1.0 PURPOSE

This protocol describes the comparison of the clinical performance of the original ABCA-GIRMS (Automated Breath Carbon Analyzer – Gas Isotope Ratio Mass Spectrometer) and new ABCA2-GIRMS systems when used to analyze ratios of $^{13}CO_2$ to $^{12}CO_2$ in breath samples collected using Cairn's ^{13}C-Spirulina GEBT, that are used to calculate $^{13}CO_2$ excretion rates (kPCD min^{-1}) that describe gastric emptying rates.

2.0 BACKGROUND

The performance characteristics of the ABCA GIRMS instrument currently in use for analyzing carbon stable isotope ratios of CO_2 in breath samples collected during administration of the GEBT were established and documented in PRO-QC-114.01, Analytical Performance of ABCA GIRMS Instruments, and the associated report (submitted as part of PMA P110015).

This protocol includes studies that seek to confirm the acceptable level of clinical performance measures of the ABCA2 GIRMS system and demonstrate comparable performance characteristics to the original ABCA GIRMS system. This study will be performed in accordance with CLSI Guidance EP12-A2, User Protocol or Evaluation of Qualitative Test Performance; Approved Guideline, Second Edition and Assay Migration Studies for In Vitro Diagnostic Devices – Guidance for Industry and FDA Staff.

3.0 RESPONSIBILITIES

Requirement	Responsibility
Protocol generation	Director of Quality Assurance and Regulatory Compliance
Protocol approval	Clinical Laboratory Director, Chief Information and Instrumentation Officer, Director of Laboratory Operations
Protocol execution	Clinical Laboratory personnel
Data analysis	Data Management Associate, Director of Laboratory Operations, Director of Quality Assurance and Regulatory Compliance, Chief Information and Instrumentation Officer
Report generation	Director of Quality Assurance and Regulatory Compliance

	NAME (Print)	SIGNATURE	DATE
Originator:			
Approved By:	Alex Ryder		9/2/20
Approved By:			
Approved By:			

CE 9-3-20

Requirement	Responsibility
Report approval	Clinical Laboratory Director, Chief Information and Instrumentation Officer, Director of Laboratory Operations
Validation package archival	Document Control

4.0 DEFINITIONS

Term or Abbreviation	Definition
ABCA GIRMS or ABCA2 GIRMS	Sercon's Automated Breath Carbon Analyzer Gas Isotope Ratio Mass Spectrometer, used to analyze stable isotopes of carbon in breath samples
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 two delta values
GEBT	Gastric Emptying Breath Test
kPCD	1000 x Percent Dose (of Carbon-13) Excreted, $^{13}CO_2$ excretion rate (min^{-1}) primary GEBT metric
Measurand	Quantity intended to be measured
NCR	Nonconformance Report
SOP	Standard Operating Procedure
QA	Quality Assurance
QC	Quality Control

5.0 TRAINING REQUIREMENTS

Operators conducting this validation must be qualified according to the position requirements and must be successfully trained in the validation protocol and all analytical method SOPs/documentation associated with execution of this protocol. Training shall be documented, and training records maintained in accordance with Cairn's requirements stated in SOP-QA-007, Training System.

6.0 EQUIPMENT/MATERIALS

A list of equipment and materials required for execution of this protocol is provided in Table 1.

Table 1. Equipment/Materials List

Equipment/Materials Description	Manufacturer/Vendor	Model Number/Comments	SPEC Numbers
ABCA GIRMS	Europa Scientific	ABCA-A	N/A
ABCA2 GIRMS	Sercon Limited	ABCA-C/ABCA-D	N/A

Equipment/Materials Description	Manufacturer/Vendor	Model Number/Comments	SPEC Numbers
Monoject Tubes	Covidien/McKesson	8881301710/48992	SPEC-QA-016
BD 10mL Syringes with BD 20G1 PrecisionGlide Needles	BD Diagnostics Systems	309604	N/A

Information regarding test materials and reference materials will be recorded at the time of testing and documented in the validation report.

7.0 PROCEDURES

7.1 Validation Requirements: Clinical Diagnostic Agreement

Determine clinical performance characteristics for ABCA2 GIRMS according to CLSI Guideline EP12 and FDA's Guidance Assay Migration Studies for In Vitro Diagnostics by analyzing 75 sets of patient breath samples on Cairn's original ABCA GIRMS and new ABCA2 GIRMS instruments.

PRO-CD-044, ABCA2 GIRMS Clinical Performance Study - Patient Sample Collection, describes the collection of patient samples for the clinical performance studies under an IRB approved protocol. Analyze breath samples collected prior to and after consumption of the GEBT test meal according to this protocol. Analyze each set of breath samples from each participant on Cairn's original ABCA GIRMS instrument and two new ABCA2 GIRMS instruments.

Determine clinical performance by comparing the diagnosis determined by kPCD values at each individual timepoint and overall diagnosis algorithm (e.g. two out of three kPCD values below the reference range cutoff point at 90, 120, and 150 minutes or T_{max} at 240 minutes) on the original ABCA GIRMS with diagnoses determined in the same way on the new ABCA2 GIRMS instruments.

Table 2. Clinical Performance Acceptance Criteria

Description	Justification
ABCA GIRMS instruments will be considered equivalent if: • ≥95% of individual diagnoses at each timepoint (45, 90, 120, 150, 180, and 240 minutes) are in agreement, and • ≥95% of overall diagnosis (by algorithm) are in agreement.	A requirement for 95% diagnostic agreement allows for the few cases where a borderline subject may fall on either side of the diagnostic 'line' (reference range COP) without significant error in the DOB.

For justification of these acceptance criteria see REP-QC-018, Validation Criteria for ABCA-GIRMS systems

7.2 General Procedures

7.2.1 Accession and analyze breath samples according to SOP-CD-021, GEBT Breath Sample Accessioning and Chain of Custody and SOP-CD-005, Breath Test Processing.

7.2.2 Prepare QC standards and reference gases for this protocol according to SOP-QC-043, QC Gas Preparation for ABCA GIRMS.

7.2.3 Run the ABCA GIRMS and ABCA2 GIRMS systems according to SOP-QC-017, Operation, Calibration and Maintenance – ABCA GIRMS and SOP-QC-090, Operation, Calibration and Maintenance – ABCA2 GIRMS.

7.2.4 Analyze breath samples first on the original ABCA-GIRMS system and then on either the ABCA-C or ABCA-D ABCA2 GIRMS system. Analyze samples a third time on the ABCA2 GIRMS system on which the samples have not yet been analyzed. Note: the original ABCA-GIRMS system, ABCA-A utilizes a larger sample of breath (~4 mL) than the ABCA2-GIRMS systems (1-2 mL). For this reason, all samples will be analyzed on ABCA-A first. The order in which samples are analyzed on ABCA-C and ABCA-D will be randomized to minimize any bias cause by running the same samples on different instruments in the same order as outlined in Table 3.

Table 3. Randomization Table

	Study Participant Breath Sample Analysis Order		
Subject Number	ABCA GIRMS (A)	ABCA2 GIRMS (C)	ABCA2 GIRMS (D)
001	1	2	3
002	1	3	2
003	1	3	2
004	1	2	3
Etc	Etc	Etc	Etc

7.2.5 Enter LAF, gas standards and subject breath sample delta values into the Linearity Adjustment Excel workbook according to SOP-QC-090 for samples analyzed on the ABCA2 GIRMS systems.

7.2.6 Enter Quality Control standards data on the appropriate QC charts which are located in the instrument laboratory. Verify that QC values are within accepted ranges according to SOP-QC-090. If QC data is outside the accepted ranges, initiate a nonconformance report (NCR) according to SOP-QA-014, Control and Handling of Nonconformances, to investigate a potential cause of the nonconformance.

7.2.7 Enter DOB and kPCD results for each patient on each instrument into a spreadsheet for analysis.

8.0 STATISTICAL ANALYSIS

8.1 Clinical Diagnostic Agreement

8.1.1 Determine the diagnosis at individual time points for each subject on each instrument as follows:

- Positive for gastroparesis: kPCD below the reference range cut-off point (Table 4) for the applicable time point
- Negative for gastroparesis: kPCD above the reference range cut-off point (Table 4) for the applicable time point

Table 4. Reference Range Cut-Off Points for ^{13}C-Spirulina GEBT

Timepoint	45 min	90 min	120 min	150 min	180 min	240 min
Reference Range COP (kPCD min^{-1})	12.9	26.9	34.4	39.5	43.0	35.0

8.1.2 Determine an overall diagnosis for each subject on each instrument using the following algorithm:

- Positive for gastroparesis if two out of three of the 90-, 120-, 150-timepoints are below the reference range cutoff point (Table 4) OR T_{max} (maximum kPCD value) occurs at the 240-minute timepoint.

Note: this algorithm is not an FDA approved algorithm for determining diagnosis by GEBT but is a convenient means of consistently determining an overall diagnosis that is supported by the interpretative guidelines published in the FDA approved labeling.

8.1.3 Determine the percent agreement in diagnosis between the comparative method (original ABCA-GIRMS) and the candidate method (new ABCA2-GIRMS) at each time point and for the overall diagnosis according to CLSI Guideline EP12-A2:

Prepare a contingency table (see Table 5) showing agreement between diagnoses between the comparative and candidate methods and each time point and for the overall diagnosis.

Table 5. Example contingency table for calculating percent agreement

Candidate Method	Comparative Method		
	Positive	Negative	Total
Positive	a	b	a+b
Negative	c	d	c+d
Total	a+c	b+d	n

Calculate overall percent agreement, percent positive agreement and percent negative agreement as follows:

- Overall percent agreement = 100 x (a+d)/n
- Percent positive agreement = 100 x a/(a+c)
- Percent negative agreement = 100 x d/(b+d)

8.1.4 Determine the 95% confidence intervals for the agreement measures according to CLSI Guideline EP12-A2 section 10.2.2.

9.0 DATA COLLECTION AND REPORTING

9.1 Collection and Review

9.1.1 Record all data according to SOP-QC-030, Data Generation and Review. Raw data or copies of data (marked as such with location of original data indicated) must be included in the validation package.

9.1.2 All data generated during execution of the validation protocol will be reviewed for completeness and accuracy in accordance with SOP-QC-030, Data Generation and Review.

9.2 Reporting

9.2.1 Collate the results of the executed validation protocol, including all associated data, in a verification report.

9.2.1.1 The report must be approved by the same individuals who approved the associated validation protocol(s).

9.2.2 Each completed validation package shall contain all relevant approved protocols, supporting data and reports.

9.2.3 Verification packages are controlled and archived by Document Control in accordance with SOP-QA-020, Quality Records.

10.0 NONCONFORMANCE

Document nonconformances according to SOP-QA-014, Handling and Control of Nonconformances.

11.0 REFERENCES

A. Assay Migration Studies for In Vitro Diagnostic Devices – Guidance for Industry and FDA Staff

B. CLSI Guideline EP12-A2, User Protocol or Evaluation of Qualitative Test Performance; Approved Guideline, Second Edition, 2008.

A. PRO-CD-044, ABCA2 GIRMS Clinical Performance Measures – Patient Sample Collection
B. REP-QC-018, Validation Criteria for ABCA-GIRMS systems
C. SOP-CD-005, Breath Test Processing
D. SOP-CD-021, GEBT Breath Sample Accessioning and Chain of Custody
E. SOP-QA-007, Training System
F. SOP-QA-014, Control and Handling of Nonconformances
G. SOP-QA-020, Quality Records
H. SOP-QC-030, Data Generation and Review
I. SOP-QC-043, QC Gas Preparation for ABCA GIRMS

J. SOP-QC-017, Operation, Calibration and Maintenance ABCA-GIRMS
K. SOP-QC-090, Operation, Calibration and Maintenance, ABCA2-GIRMS

12.0REVISION HISTORY

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

20.0 EXHIBIT C. PRO-QC-191.00, ABCA2 GIRMS ANALYTICAL VALIDATION – BIAS AND TOTAL ANALYTICAL ERROR

Effective Date: SEP 03 2020

1.0 PURPOSE

The purpose of this bias and total analytical error analytical validation study is to establish documented evidence toward the safety and effectiveness of the ABCA2 GIRMS system to be used to determine the carbon stable isotope ratio (expressed as $\delta^{13}C$) of CO_2 in breath samples collected during administration of Cairn's Gastric Emptying Breath Test (GEBT).

2.0 BACKGROUND

The performance characteristics of the ABCA GIRMS instrument currently in use for analyzing carbon stable isotope ratios of CO_2 in breath samples collected during administration of the GEBT were established and documented in PRO-QC-114.01, Analytical Performance of ABCA GIRMS Instruments, and the associated report (submitted as part of PMA P110015).

This protocol includes studies that seek to confirm an acceptable level of bias and total analytical error of the ABCA2 GIRMS system and demonstrate comparable performance characteristics to the original ABCA GIRMS system. This study will be performed in accordance with CLSI Guidance EP09c, "Measurement Procedure Comparison and Bias Estimation Using Patient Samples"; 3rd edition; June 2018, EP21- "Evaluation of Total Analytical Error for Quantitative Medical Laboratory Measurement Procedures – 2nd Edition"; July 2016 and Assay Migration Studies for In Vitro Diagnostic Devices – Guidance for Industry and FDA Staff.

3.0 RESPONSIBILITIES

Requirement	Responsibility
Protocol generation	Data Management Associate
Protocol approval	Director of Quality Assurance and Regulatory Compliance, Clinical Laboratory Director, Chief Information and Instrumentation Officer
Protocol execution	Clinical Laboratory personnel, Data Management Associate, Chief Information and Instrumentation Officer

	NAME (Print)	SIGNATURE	DATE
Originator:	Shane Crabtree		9/2/2020
Approved By:	Samantha Pawlik		9/2/20
Approved By:	CATHERINE WILLIAMS		9-2-2020
Approved By:	See Attached		9-3-20

Effective Date:

1.0 PURPOSE

The purpose of this bias and total analytical error analytical validation study is to establish documented evidence toward the safety and effectiveness of the ABCA2 GIRMS system to be used to determine the carbon stable isotope ratio (expressed as $\delta^{13}C$) of CO_2 in breath samples collected during administration of Cairn's Gastric Emptying Breath Test (GEBT).

2.0 BACKGROUND

The performance characteristics of the ABCA GIRMS instrument currently in use for analyzing carbon stable isotope ratios of CO_2 in breath samples collected during administration of the GEBT were established and documented in PRO-QC-114.01, Analytical Performance of ABCA GIRMS Instruments, and the associated report (submitted as part of PMA P110015).

This protocol includes studies that seek to confirm an acceptable level of bias and total analytical error of the ABCA2 GIRMS system and demonstrate comparable performance characteristics to the original ABCA GIRMS system. This study will be performed in accordance with CLSI Guidance EP09c, "Measurement Procedure Comparison and Bias Estimation Using Patient Samples"; 3rd edition; June 2018, EP21- "Evaluation of Total Analytical Error for Quantitative Medical Laboratory Measurement Procedures – 2nd Edition"; July 2016 and Assay Migration Studies for In Vitro Diagnostic Devices – Guidance for Industry and FDA Staff.

3.0 RESPONSIBILITIES

Requirement	Responsibility
Protocol generation	Data Management Associate
Protocol approval	Director of Quality Assurance and Regulatory Compliance, Clinical Laboratory Director, Chief Information and Instrumentation Officer
Protocol execution	Clinical Laboratory personnel, Data Management Associate, Chief Information and Instrumentation Officer

	NAME (Print)	SIGNATURE	DATE
Originator:			
Approved By:	Alex Ryder		9/2/20
Approved By:			
Approved By:			

ce 9-3-20

Requirement	Responsibility
Data analysis	Data Management Associate, Chief Information and Instrumentation Officer, Director of Laboratory Operations
Report generation	Data Management Associate
Report approval	Director of Quality Assurance and Regulatory Compliance, Clinical Laboratory Director, Chief Information and Instrumentation Officer
Validation package archival	Document Control

4.0 DEFINITIONS

Term or Abbreviation	Definition
ABCA GIRMS or ABCA2 GIRMS	Sercon's Automated Breath Carbon Analyzer Gas Isotope Ratio Mass Spectrometer, used to analyze stable isotopes of carbon in breath samples
ATE	Allowable Total Error
Bias	Difference between the expectation of the test results and an accepted reference value; estimate of a systematic measurement error
Delta values ($\delta^{13}C$)	The ratio of $^{13}CO_2/^{12}CO_2$ in each breath specimen as measured by GIRMS unites permil (‰)
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
Measurand	Quantity intended to be measured
NCR	Nonconformance Report
SOP	Standard Operating Procedure
TAE	Total Analytical Error
QA	Quality Assurance
QC	Quality Control

5.0 TRAINING REQUIREMENTS

Operators conducting this validation must be qualified according to the position requirements and must be successfully trained in the validation protocol and all analytical method SOPs/documentation associated with execution of this protocol. Training shall be documented, and training records maintained in accordance with Cairn's requirements stated in SOP-QA-007, Training System.

6.0 EQUIPMENT/MATERIALS

A complete list of all equipment and instrumentation required for execution of this protocol is provided in attached analytical procedures SOP-QC-017, Operation, Calibration and Maintenance – ABCA GIRMS and SOP-QC-090, Operation, Calibration and Maintenance – ABCA2 GIRMS. A list of major equipment/materials is provided in Table 1 below.

Information regarding test materials and reference materials will be recorded at the time of testing and documented in the validation report.

Table 1. List of Equipment/Materials

Equipment Description	Manufacturer/Vendor	Model Number/Comments
ABCA GIRMS	Europa Scientific	ABCA-A
ABCA2 GIRMS	Sercon Limited	ABCA-C/ABCA-D
Exetainer Tubes	Labco Limited	438W/NP (white cap) 438B/NP (blue cap)
Monoject Tubes	Covidien/McKesson	8881301710/48992

7.0 PROCEDURES

7.1 Validation Requirements

Determine bias and total analytical error using delta over baseline (DOB) values, i.e. differences in delta values ($\delta^{13}C$) of breath samples collected at any given timepoint and the baseline/pre-meal breath delta value) obtained by the ABCA GIRMS systems (original ABCA GIRMS and two new ABCA2 GIRMS systems. DOB values are used together with patient demographic information to calculate kPCD values. kPCD is the metric used to describe gastric emptying rates and make diagnostic decisions (refer to PRO-QC-202, ABCA2 GIRMS Clinical Performance Measures for determining diagnostic equivalence between the ABCA GIRMS instruments). DOB values are direct measures of ABCA GIRMS instruments' output (kPCDs include patient demographic information which would confound the analysis) and therefore DOBs are used to determine instrument bias/total analytical error.

PRO-CD-044, ABCA2 GIRMS Clinical Performance Measures – Patient Sample Collection, describes the collection of patient samples for clinical performance measuring studies, under an IRB approved protocol. Collect a minimum of seventy-five (75) sets of breath samples and conduct clinical performance measures experiments according to PRO-QC-202, ABCA2 GIRMS Clinical Performance Measures, by analyzing patient breath samples on the ABCA GIRMS system first and then on the ABCA2 GIRMS systems (ABCA-C or ABCA-D), then a third time on the ABCA2 GIRMS system on which the samples have not yet been analyzed.

7.1.1 Bias

Perform Bias calculations according to CLSI guidance EP09c and FDA's Guidance, Assay Migration Studies for In Vitro Diagnostic Devices. Refer to section 8 for a description of the statistical analysis of the data.

7.1.2 Total Analytical Error

Perform Total Analytical Error calculations according to CLSI guidance EP21 and FDA's Guidance, Assay Migration Studies for In Vitro Diagnostic Devices. Refer to section 8 for a description of the statistical analysis of the data.

Table 2. Bias and Total Analytical Error Acceptance Criteria

Description	Justification
The Bias in DOBs on ABCA2-GIRMS should not exceed ±0.5‰ from the results from ABCA-A.	Previously approved instrument accuracy/bias requirements and manufacturer's specification for accuracy of ABCA-GIRMS.
Total Analytical Error in DOBs shall not exceed 30%.	Based on biologic variability data used to calculate a total allowable error (ATE) goal.

For justification of these acceptance criteria see REP-QC-018, Validation Criteria for ABCA-GIRMS systems

7.2 General Procedures

See PRO-QC-202.00 ABCA2-GIRMS Clinical Performance Measures, Section 7.2 for a description of general procedures for collecting data to be used in this study.

8.0 STATISTICAL ANALYSIS

8.1 Bias Comparison

Compare bias in patient samples according to EP09c – Third Edition Measurement Procedure Comparison Bias Estimation Using Patient Samples.

- Calculate the difference in DOBs, between the candidate method (ABCA-C or ABCA-D) to the comparative method (ABCA-A).
- Calculate the mean difference and the standard error (calculated by dividing the standard deviation by the square root of the number of samples) of the difference.
- Calculate the 95% confidence interval and compare results to the acceptable limit of ±0.5‰ DOBs. If the interval is within this acceptance limit the results are deemed acceptable.

8.2 Method Comparison

Compare results from the two ABCA2 instruments (ABCA-C and ABCA-D) to the original ABCA (ABCA-A) by using a mixed effects regression model in which the DOB values of the ABCA2 machines are predicted using the ABCA DOB values.

The mixed effect model follows the model:

$$\boldsymbol{y} = \boldsymbol{X\beta} + \boldsymbol{Zu} + \boldsymbol{\epsilon}$$

Where:

$\boldsymbol{y}$ is the known vector of DOB values from ABCA2 (DOB of ABCA-C/D values
$\boldsymbol{X}$ is a vector of the observations of the fixed effects (DOB A values)
$\boldsymbol{\beta}$ is a vector of the fixed effects coefficients (DOB A values)
$\boldsymbol{Z}$ is a vector of observations of the random effects (patient ID)
$\boldsymbol{u}$ is a vector of random effects values (patient ID)
$\boldsymbol{\epsilon}$ is a vector of error values (residuals)

After the regression equation has been established, calculate the confidence intervals for slope and the intercept. Once the confidence intervals have been calculated, determine the Bias at the following DOB values:

DOB
0
5
10
15
20
25

Once the bias has been calculated, compare the bias against the acceptance criteria. If the bias is less than the ±0.5‰ acceptance criterion then the comparison is considered acceptable.

8.3 Total Analytical Error

Calculate Total Analytical Error (TAE) according to EP21 – Second Edition, Evaluation of Total Analytical Error for Quantitative Medical Laboratory Measurement Procedures. Use DOBs obtained during PRO-QC-202 – ABCA2-GIRMS Clinical Performance Measures. Calculate TAE by comparing DOB results from the original ABCA GIRMS system to DOB results from the two new ABCA2 GIRMS system.

- Determine the TAE estimate using a total of 75 patients with 8 breath samples collected for each patient (2 pre-meal samples, a 45, 90, 120, 150,180 and 240-minute timepoints). Obtain appropriate DOB values for each patient and use these values to calculate the total analytical error.

- To calculate the TAE first calculate the difference value (d_i) by subtracting the DOB results from ABCA-A from the DOB results from the comparative method (either ABCA-C or ABCA-D).

$$d_i = Candidate_{\ i} - \frac{\sum_{j=1}^{R} Comparative_{i,j}}{R}$$

Where j is the replicate value from 1 to R for the ith patient (i = 1 to n)

Express d_i as a percent:

$$\%d_i = \frac{d_i}{\frac{\sum_{j=1}^{R} Comparative_{i,j}}{R}} * 100$$

Sort all $\%d_i$ from lowest to highest and calculate the Low and High Rank Positions to determine the TAE limits.
For 95%:

$$Low\ Rank\ Position = 0.5 + n * 0.025$$
$$High\ Rank\ Position = 0.5 + n * 0.975$$

Using these positions, find the $\%d_i$. If the rank position is nonintegral, interpolate the % difference from the values bracketing the rank position.

If the low TAE limit is greater than or equal to 30% and the high TAE limit is less than or equal to 30% then the minimum performance goal has been met.

9.0 DATA COLLECTION AND REPORTING

9.1 Collection and Review

9.1.1 Record all data according to SOP-QC-030, Data Generation and Review. Raw data or copies of data (marked as such with location of original data indicated) must be included in the validation package.

9.1.2 All data generated during execution of the validation protocol will be reviewed for completeness and accuracy in accordance with SOP-QC-030, Data Generation and Review.

9.2 Reporting

9.2.1 Collate the results of the executed validation protocol, including all associated data, in a verification report.

9.2.2 The report must be approved by the same individuals who approved the associated validation protocol(s).

9.2.3 Each completed validation package shall contain all relevant approved protocols, supporting data and reports.

9.2.4 Verification packages are controlled and archived by Document Control in accordance with SOP-QA-020, Quality Records.

10.0 NONCONFORMANCE

Document nonconformances according to SOP-QA-014, Handling and Control of Nonconformances.

11.0 REFERENCES

A. Assay Migration Studies for In Vitro Diagnostic Devices – Guidance for Industry and FDA Staff
B. CLSI Guidance EP09c, Measurement Procedure Comparison and Bias Estimation Using Patient Samples; 3rd edition; June 2018
C. EP21- Evaluation of Total Analytical Error for Quantitative Medical Laboratory Measurement Procedures – 2nd Edition
D. PRO-CD-044, ABCA2 GIRMS Clinical Performance Measures – Patient Sample Collection
E. PRO-QC-067, Verification of Values Assigned to Reference Materials Used in Analyses on the ABCA and ANCA Instruments)
F. PRO-QC-202, ABCA2 GIRMS Clinical Performance Measures
G. REP-QC-018, Validation Criteria for ABCA-GIRMS systems
H. SOP-QA-007, Training System
I. SOP-QA-014, Control and Handling of Nonconformances
J. SOP-QA-020, Quality Records

K. SOP-QC-017, Operation, Calibration and Maintenance – ABCA GIRMS
L. SOP-QC-030, Data Generation and Review
M. SOP-QC-043, QC Gas Preparation for ABCA GIRMS
N. SOP-QC-090, Operation, Calibration and Maintenance – ABCA2 GIRMS

12.0 REVISION HISTORY

Revision Level	Brief Description of Revision(s)	Effective Date
00	Initial document	Refer to first page of this procedure

21.0 EXHIBIT D: COVID-19 TRANSMISSION MITIGATION PLAN

COVID-19 TRANSMISSION MITIGATION PLAN

This COVID-19 transmission mitigation plan and questionnaire (Exhibit E) is to be utilized by Cairn Diagnostics when participating as a trial site. The sub-investigators associated with the execution of this protocol will use their COVID-19 transmission mitigation procedures.

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.

Visit 2: 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.

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 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 3: 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 is performed via a phone call or telecommunication platform, a memo noting the follow up will be placed in the participant's file.

22.0 EXHIBIT E: 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

23.0 EXHIBIT F. 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.