Clinical Study to Confirm Safety and Accuracy in Detection of H. pylori with ^{13}C-Urea Breath Test using the BreathID® Hp and BreathID® Hp Lab Systems in the Pediatric Population

Protocol No. PED-HP-0616B

Document No.: CSD00092	Total Pages: 59
Protocol version: 2.0	Last update on: Mar 12, 2017

	Name	Position
Author	Avraham Hershkowitz	Clinical Trials Manager
Reviewed by	Gil Guggenheim	Clinical Research Development Manager
Reviewed by	Michal Brunner	Clinical Trials Manager
Approved by	Melina Arazy, MD	VP of Clinical Affairs
Electronic signatures and approvals, effective information and Lifecycle Status can be obtained by accessing the system generated approval page associated with this document. The system generated approval page can be retrieved from the PDM system.		

This publication, in whole or in part, may not be reproduced, disclosed, translated into any language or computer language or distributed without the prior written consent of:

Exalenz Bioscience Ltd. 4 Ha'Maayan St. Modiin, Israel 7177872

Versions Control:

Version	Date	Responsible Person	Description of Change
1	20 Sep 2016	Avraham Hershkowitz	Initial Release Israel MOH Stool and EGD
1.1	19 Sep 2016	Michal Brunner	Internal version – administrative changes, Stool and EGD (not released)
1.2	20 Sep 2016	Michal Brunner	Initial US Release for IDE
1.3	13 Nov 2016	Avraham Hershkowitz	Minor changes due to IDE conditional approval-
2.0	12 Mar 2017	Gil Guggenheim	Clarifying the Blinding of PI and minor edits (Post Pre-Submission Comments Q170072)

TABLE OF CONTENTS

ABBREVIATIONS

ACG	American College of Gastroenterology
AE	Adverse Event
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological
CFR	Code of Federal Regulation
CP	Conditional Power
CRF	Case Report Form
DOB	Delta over Baseline
EC	Ethics Committee
EGD	Esophagogastroduodenoscopy
EIA	Enzyme ImmunoAssay test
ELISA	Enzyme-linked immunosorbent assay
FA	Full Analysis (cohort)
FAT	Fecal Antigen Test
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GERD	Gastroesophageal Reflux Disease
GI	Gastrointestinal
H. pylori	Helicobacter pylori
HP	Helicobacter pylori
ICA	Immuno-Chromatographic Assays
ICF	Informed Consent Form
ICH	International Conference of Harmonization
IDE	Investigational Device Exemption
IEC	Independent Ethics Committee
IND	Investigational New Drug
IRB	Institutional Review Board
IVD	In Vitro Diagnostics
MD	Medical Doctor
NDA	New Drug Application

PP	Per Protocol
PPI	Proton Pump Inhibitor
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SF	Screen Failure
SUSAR	Suspected Unexpected Serious Adverse Reaction
UADE	Unanticipated Adverse Device Effect
UBT	^{13}C-Urea Breath Test
US	United States (of America)
USA	United States of America

1. PROTOCOL SYNOPSIS

Protocol Title:	***Clinical Study to Confirm Safety and Accuracy in Detection of H. pylori with ^{13}C-Urea Breath Test using the BreathID® Hp and BreathID® Hp Lab Systems in the Pediatric Population***
Short Title:	Pediatric Hp study
Protocol Reference #:	PED-HP-0616B
Version and Date:	Protocol v 2.0, March 12, 2017
Phase of Development:	Phase III
Sponsor and Study Monitor:	**Exalenz Bioscience Ltd.** 4 Ha'Maayan Street Modi'in, Israel 7177872 Tel: +972-8-9737500 Fax: +972-8-9737501
Investigated Disease:	*Helicobacter pylori* infection
Study Start Date:	Q2 2017
Duration of Study:	Approximately 6 months
Investigational Products (IP):	***BreathID® Hp System*** (K130524) and ***BreathID® Hp Lab System*** (K162150)
IP Details:	1. The ***BreathID® Hp System*** (K130524) that uses the approved test kit ***IDkit:Hp™ One*** in adults, comprised of a nasal cannula and the approved ^{13}C-urea/citric acid reagent (NDA-21-314). 2. The ***BreathID® Hp Lab System*** (K162150) that uses thecleared test kit ***IDkit:Hp™ Two***, comprised of two breath sample bags and the approved ^{13}C-urea/citric acid reagent (NDA-21-314).

Study Goal and Rationale:

The goal of this study is to expand the use of the breath test to the pediatric population for safety at the dose being tested, and effectiveness, to gain indication of the test kits in pediatrics. Also, this study will address CDER's request for expansion of the use of the ^{13}C-urea and citric acid that is the subject of NDA-21-314, to the pediatric population (ages 3-18[1]).

Based on a recent guidance document released by the FDA on June 21, 2016: *Leveraging Existing Clinical Data for Extrapolation to Pediatric Uses of Medical Devices*, the ***BreathID® Hp System*** and the ***BreathID® Hp Lab System***, meet the main criteria needed for extrapolation of the efficacy of clinical data for Pediatric Use:

a) **Relevancy of Adult Data and Similarity of Response:** The breath test, its kits and the contained test substrate are all well established in the adult population (K130524 and K162150) using the biopsy composite reference method as the gold standard. The *H. pylori* infection is the same in children and adults as *H. pylori* colonize the stomach in humans of all ages. In fact, infection is commonly spread to children in the family. The mechanism of detection for this breath test is by measuring the activity of the *H. pylori* organisms that split urea and are only present in the human stomach. Positive breath testing is indicative of *H. pylori* activity in the subject's stomach, independently of the subject's age. This has been confirmed by individual investigators and other companies (PMA# P1000254) utilizing the composite reference standard[(1-3)].

b) **Quality of Safety and Effectiveness Data:** The clinical data collected for the clearance of the ***BreathID® Hp***

[1] Subjects 18 years of age and older will not be included in this study. The age range of 18-21 wasincluded in the framework of the adult protocols (any adult over 18) to support this cleared indication in the adult population.

System and the ***BreathID® Hp Lab System*** breath tests (K130524 and K162150 respectively), were collected in accordance with the ethical principles under Investigational Review Board (IRB) approval consistent with Good Clinical Practice (GCP) and with the applicable regulatory requirements.

This approach was confirmed by FDA through Pre-Sub Q170072.

This study is thus primarily aimed to confirm tolerance and safety of the dose used for the pediatric population. The ^{13}C-urea and citric acid (NDA-21-314) are contained in ***IDkit:Hp™ One*** in the ***BreathID® Hp System*** (K130524) and ***IDkit:Hp™ Two*** in the ***BreathID® Hp Lab System*** (K162150). Additionally the respective means of breath collection, the nasal cannula and breath collection bags contained in the respective kits, will be confirmed for pediatric use.

Although it is believed that the efficacy of the test can be fully extrapolated from the adult data, subject's *H. pylori* status will also be collected as part of this study in order to further support and confirm the efficacy of the breath test in the pediatric population.

The safety of the specific drug substance in children at the dose utilized in the test setting remains to be established in this study. The dose has been proven to be both safe and effective in adults in clinical trials as well as in routine clinical use in more than 150,000 subjects.

Upper endoscopy - Esophagogastroduodenoscopy (EGD) is generally not standard of care for evaluation of children with symptoms associated with *H. pylori* infection. As such, it is difficult to justify performing the procedure which requires sedation only for the research purpose. Children are evaluated for *H. pylori* infection most commonly with stool antigen

testing. Therefore, this study proposes to test the drug and its dosing in symptomatic children suspected of having *H. pylori* who will undergo both a breath test and an FDA cleared stool test. The stool test has a sensitivity and specificity ranging from 90 to 97%, and comparing the breath test to the stool test results will confirm that the use of this test is reasonable in the target population.

This study will enroll all-comers whether for initial diagnosis or post-eradication testing. It will predominantly (approximately 2/3) enroll children under the age of 12 to confirm drug/dose tolerance and confirm its efficacy. Older children between 12 and 18 years are similar to adults and no more than a third (approximately 1/3) will be enrolled from this age group.

Description of Test:

The ^{13}C-Urea Breath Test is performed using a test kit that contains 75mg of non-radioactive ^{13}C-urea and citric acid to be dissolved in tap water ("solution").

Exhaled breath will be collected using:

(1) a nasal cannula for continual passive collection of breath samples from the nose before and after ingestion of the solution, and/or

(2) two breath sample bags (orally and actively exhaled breath) obtained at two defined time points, before and after ingestion of the solution.

Both exhaled breath collection methods will be performed in parallel, and the subject will ingest the solution only once after baseline breath collection.

The subject's exhaled breath is analyzed and the ratio between $^{13}CO_2$ and $^{12}CO_2$ is computed.

The ***BreathID® Hp System*** using the cleared ***IDkit:Hp™ One*** collects breath passively from the subject's nostrils via a nasal cannula whose other end is connected to

the device via a luer lock connector.

The ***BreathID® Hp Lab System*** is a modified version of the ***BreathID® Hp System,*** using ***IDkit:Hp™ Two*** enabling up to 10 pairs of bags (i.e., 2 bags each from 10 separate patient sampling events, resulting in 10 tests) to be measured consecutively and automatically by means of attaching the bags to a dedicated ***Auto Sampler*** unit. The bags will be collected and measured by the ***BreathID® Hp Lab System*** within 14 days from the time of collection.

When using breath sample bags, the initial sample, collected immediately before ingesting the ^{13}C-urea/citric acid solution, defines the baseline ratio of $^{13}CO_2$ to $^{12}CO_2$. The second sample, collected 15 to 20 minutes following the ^{13}C-urea/citric acid solution ingestion, is analyzed to determine changes in the $^{13}CO_2/^{12}CO_2$ ratio.

For both the ***BreathID® Hp System*** and the ***BreathID® Hp Lab System,*** the patient is considered *H. pylori* positive when the difference between the ratios exceeds a predefined and previously cleared threshold (in adults) of 5 delta over baseline (DOB).

Comparator Details: Efficacy of the test can be fully extrapolated from the adult data (as confirmed with FDA through Pre-Submission Q170072). The goal of this study is thus primarily aimed to support expansion of the use of the ^{13}C-urea and citric acid that is the subject of NDA-21-314, to the pediatric population (ages 3-18) and to confirm tolerance of the dose used for this target population. The ^{13}C-urea and citric acid is contained in ***IDkit:Hp™ One*** (K130524) in the ***BreathID® Hp System*** and ***IDkit:Hp™ Two*** (K162150) in the ***BreathID® Hp Lab System.*** In addition to safety data that is collected for this study, their *H. pylori* status using the stool antigen test will also be collected as a comparator in order to further support

and confirm the efficacy of the breath test.

As mentioned above, EGD is not standard of care for evaluation of children with symptoms associated with *H. pylori* infection. They are evaluated for *H. pylori* infection most commonly with stool antigen testing. This study proposes to test the drug and its dosing in symptomatic children suspected of having *H. pylori* who will undergo both a breath test and an FDA cleared stool test. This population is chosen as they are the target population for the breath test and therefore drug use, and their evaluation with the stool test, will confirm that the use of this test is reasonable in the target population. Stool testing detects *H. pylori* antigen in stool specimens and can be used for diagnosis, therapeutic monitoring, and proof of eradication post treatment.

This study will use the Premier Platinum HpSA PLUS (K053335) Enzyme Immunoassay test kit for the detection of *H. pylori* antigen in human stool specimens (Appendix B). It will be tested in a central clinical laboratory.

The stool enzyme immunoassay employs a mixture of monoclonal anti-*H. pylori* antibodies as capture antibody and a mixture of peroxidase-conjugated monoclonal anti-*H. pylori* antibodies as detection antibody. Based on the intensity of color developed, results are reported as *H. pylori* antigen detected, not detected, or indeterminate.

A positive result (antigen detected) is indicative of *H. pylori* infection. A negative result (antigen not detected) indicates absence of *H. pylori* or an antigenic level below the assay limit of detection.

Study Design:

Multi-center, non-randomized, open label study, designed to confirm the safety and the efficacy of ^{13}C-urea breath test using the ***BreathID® Hp and BreathID® Hp Lab Systems, as appropriate,*** in pediatric subjects and confirm the efficacy of the respective means of breath collection, the nasal cannula

and breath collection bags, in this population compared to *stool antigen* testing. A central lab will analyze the stool specimens. Children that cannot inflate the breath collection bags adequately may perform the UBT only with nasal cannula. All children will be exposed to the same dose of the non-radioactive ^{13}C-urea and citric acid.

Subjects will be enrolled on a walk-in basis. Subjects will be treated only based on the established methods used in the local routine clinical practice. No patient management decisions should be made based on the investigational ***BreathID® Hp*** and/or ***BreathID® Hp Lab System*** test outcomes and the investigator and treating physician will remain blinded to the ***BreathID® Hp*** and/or ***BreathID® Hp Lab System*** test results until the end of the study.

Study Population: Consecutive pediatric subjects aged between 3 and 18 years evaluated by the physician, or presented by referral from other institutions or physicians who are clinically indicated to undergo *H. pylori* testing and meet study criteria will be enrolled. Distribution of subject's ages will be monitored to assure all age ranges are represented, while predominantly (approximately 2/3) enrolling children under the age of 12 to confirm drug/dose tolerance and confirm its efficacy.

Consent to participate in the study and to undergo the breath test procedures must be obtained from the legal representative/guardian of every subject following a thorough explanation of the study, including the benefits and risks (if any). The majority (approximately 2/3) of the subjects will be from US centers. Assent will also be obtained from those subjects above the age that local regulations require consenting/assenting of the child.

Sites: Up to 8 US sites will participate in this study. Non-US sites may be included.

Primary Endpoint:
Safety Endpoint:

Adverse event rate: The safety of the ^{13}C-urea/citric acid solution has been previously established in adults and in children in literature[1-3]. Any adverse event (whether related or not) will be recorded. There are no safety concerns and no adverse events anticipated for the breath test or the stool antigen test beyond those already listed in the respective labels.

Secondary Endpoint:
Efficacy Endpoints:

I. Overall agreement in diagnosis (presence/absence of *H. pylori*) between the ***BreathID® Hp System*** results where breath is collected using ***IDkit:Hp™ One*** via a nasal cannula applying the 5 DOB threshold and stool antigen outcome in the pediatric population.
II. Overall agreement in diagnosis (presence/absence of *H. pylori*) between the ***BreathID® Hp Lab System*** results when analyzing ***IDkit:Hp™ Two*** breath sample bags using the 5 DOB threshold and the stool antigen outcome in the pediatric population.

Inclusion Criteria:

To be eligible to participate in this study, subjects must meet the following inclusion criteria:

- Be older than 3 and younger than 18 years of age
- Present with a clinical indication compatible with *H. pylori* based on the judgement of the treating physician (such as abdominal pain, nausea, diarrhea, reflux, peptic ulcer, dyspepsia, etc., or following treatment for *H.pylori*)
- Subject/Legal guardian (and subject whenever relevant) is willing to sign the Informed Consent/Assent Form
- Naïve to *H. pylori* treatment in the past 6 weeks

Exclusion Criteria:

To be eligible to participate in this study, subjects must NOT meet any of the following exclusion criteria:

- Participation in other interventional trials
- PPI or H_2 blockers within two (2) weeks prior to breath test/stool antigen test
- Pregnant or breastfeeding female
- Allergy to test substrates
- Antibiotics (not related to *H. pylori* eradication) and/or Bismuth preparations within four (4) weeks prior to breath test
- Exposure to any type of anesthesia, analgesics or sedation 24 hours prior to the breath test.
- Exposure to any ^{13}C-enriched substance 24 hours prior to the breath test.
- Children 12 years and older – to be excluded after a written notification from the sponsor is received at the site that the limit of 1/3 of the sample size was achieved for this group
- Subjects outside US - to be excluded after a written notification from the sponsor is received at the site that the limit of 1/3 of the sample size was achieved for this group

Exclusion on the day of the UBT: Subject did not fast for the last hour prior to the UBT

Statistical Considerations: This study is designed to confirm safety for the use of the ^{13}C-urea/citric acid solution used in the cleared Exalenz UBT testing using the ***IDkit:Hp™ One*** and/or ***IDkit:Hp™ Two*** as part of ***BreathID® Hp System*** and/or ***BreathID® Hp Lab System*** and to confirm that they have performance measures in the pediatric population similar to that of adults. Due to the fact that esophagogastroduodenoscopy (EGD) is uncommon in pediatrics, the comparator to be used in this trial is stool antigen testing, a common test method used to diagnose and/or confirm eradication of *H. pylori* infection in

pediatrics.

Sample Size:

The sample size for this study of pediatric subjects with indications for *H. pylori* testing is calculated to allow confirmation of both, the primary and secondary study endpoints. For the safety assessment, with no related SAE's expected, a sample size of 35 evaluable subjects would provide an upper confidence limit with less than or equal to 10% for the 95% two sided confidence limit. In order to support the efficacy endpoints a sample size is calculated through the width of the two-sided 95% confidence interval of the percent overall agreement, such that the lower limit of the confidence interval will be greater than or equal to 75% with a point estimate of 90%.

A total of at least 40 evaluable subjects with no less than 5 positive subjects will be accrued for both breath collection methods. If the minimum of 5 positive subjects is not reached within the first 40 subjects enrolled, enrolment will continue until this minimal number of positive subjects is reached (with evaluable UBT results for both breath collection methods).

This will be sufficient to confirm the safety and the utility of the ***BreathID® Hp System*** and ***BreathID® Hp Lab System*** in detecting *H. pylori* infection in pediatric subjects.

Distribution of subject's ages will be monitored to assure all age ranges are represented, while at least two thirds (2/3) of the enrolled children will be under the age of 12.

Site Termination:

A study site may be terminated after appropriate consultation between the study sponsor and the principal investigator. Conditions warranting termination include, but are not limited to:

- Failure of the investigator to enroll subjects at an acceptable rate
- Insufficient adherence to protocol requirements

- Decision by the study sponsor to suspend or discontinue development of the device and/or kit
- Failure to adhere to GCP
- Sponsor decision

Regulatory Considerations:

The current clinical study will require an IDE as the subject receives a drug substance approved for adults but not yet approved for those under 18 years of age.

Study Procedure:

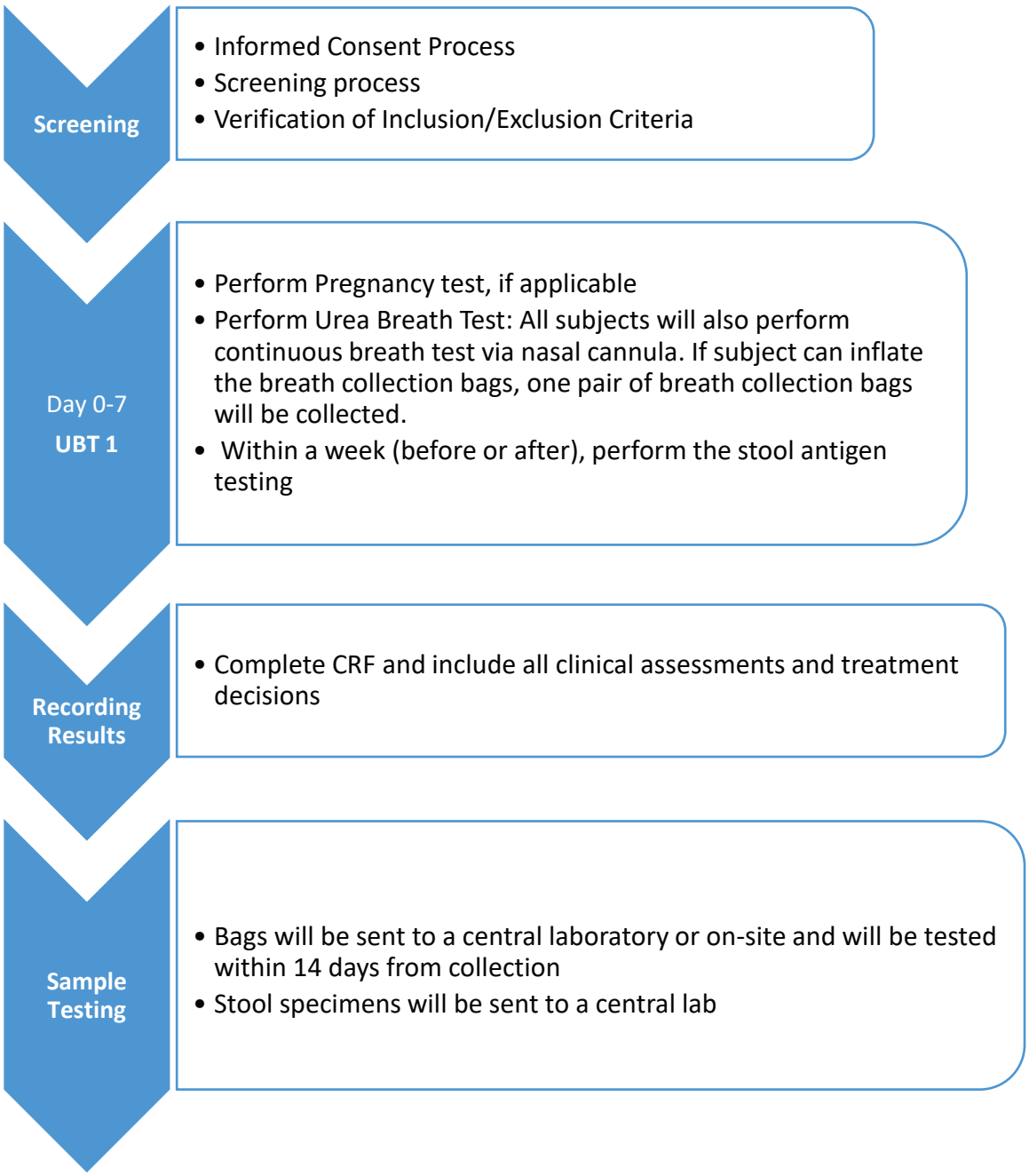
The clinical study will be conducted in compliance with this protocol and in accordance with the ethical principles under Investigational Review Board (IRB) and Ethics Committee (EC) approvals consistent with Good Clinical Practice (GCP) and with the applicable regulatory requirements.

- The investigator(s) at each site will sign a Protocol Approval Page (**Appendix A**).
- Patient management will not be affected by the ***BreathID® Hp*** and/or ***BreathID® Hp Lab System*** test results and will be based only on the common clinical method used. The investigator and treating physician will remain blinded to the breath test results until the end of the study.
- Physical examination of the subject and recording of demographic data will be completed.
- The stool antigen test will be performed for each subject after at least 2 weeks of refraining from proton pump inhibitors, antimicrobials and bismuth preparations. The test procedure is described in **Appendix B**.
- Within 1 week before or after the stool antigen test and after refraining at least 2 weeks from proton pump inhibitors and 4 weeks from antimicrobials and bismuth preparations, the subjects will perform the UBT by filling two ***IDkit:Hp™ Two*** breath collection bags, one at each time point (pre-ingestion and 15-20 minutes post-test solution ingestion) and by having breath collected via the ***IDkit:Hp™ One*** nasal cannula. Subjects unable to fill the breath collection bags will perform the breath test with the nasal

cannula only.

- All bags will be analyzed by a ***BreathID® Hp Lab System*** within 14 days of collection by unmasked personnel at the site or by a selected central lab.
- The breath collected via nasal cannula will be analyzed continually by an on-site ***BreathID® Hp System*** by unmasked personnel.
- Stool specimens will be sent to a central lab. The site coordinator will provide each subject with all supplies needed for collection of the stool specimen. The specimen will be either collected on-site, brought back to the site, brought to a local approved stool collection facility or it may be sent directly to a central laboratory from the subject's residence. A requisition form will be completed. After confirming that each stool antigen sample was sent to the central lab, the study coordinator will complete an entry line in a log documenting the shipment.
- An electronic Case Report Form (CRF) will be completed for each subject. Any additional diagnostic testing performed for the subjects will be recorded.
- Breath test results will remain non-accessible to the investigator. Unmasked site personnel will be trained to assure results are not shared.

2. VISIT SCHEDULE OVERVIEW



3. STUDY SPONSOR

Exalenz Bioscience Ltd.
Address: 4 Ha`Maayan St., Modi'in, 7177872, Israel
Tel: +972−8−9737500
Fax: +972−8−9737501
Email: contact@exalenz.com

US Address:
Exalenz Bioscience Inc.
Attn: Raffi Werner, CEO
35 Colby Ave. Suite #9
Manasquan, NJ 08736, USA
Tel: 732-722-8736

Fax: 732-722-8738
Email: raffiw@exalenz.com

Each Investigator will sign the protocol approval page (see **Appendix A**) before study initiation at their respective site.

4. BACKGROUND

Helicobacter pylori (*H. pylori*) infections remain a prevalent chronic problem worldwide. Although the prevalence of this infection appears to be decreasing in many parts of the world, *H. pylori* remains an important factor linked to the development of peptic ulcer disease, gastric malignancy and dyspeptic symptoms.(4) Whether to test for *H. pylori* in subjects with functional dyspepsia, gastro-esophageal reflux disease (GERD), subjects taking non-steroidal anti-inflammatory drugs, with iron deficiency anemia, or who are at greater risk of developing gastric cancer remains controversial. According to the *American College of Gastroenterology* (ACG) Guidelines(4) *H. pylori* can be diagnosed by endoscopic or non-endoscopic methods. A variety of factors including the need for endoscopy, pretest probability of infection, local availability of testing, and an understanding of the performance characteristics and cost of the individual tests influences the choice of evaluation in a given patient. Testing to prove eradication should be performed in subjects who receive eradication therapy for *H. pylori* for peptic ulcer disease, individuals with persistent dyspeptic symptoms despite the test-and-treat strategy, those with *H. pylori*-associated mucosa-associated lymphoid tissue (MALT) lymphoma, and individuals who have undergone resection of early gastric cancer.(4)

Children differ from adults with respect to *H. pylori* infection in terms of the prevalence of the infection, the complication rate, the near-absence of gastric malignancies, age-specific problems with diagnostic tests and drugs, and a higher rate of antibiotic resistance[2]. None of these differences are related to the basis of the breath testing. The pathophysiology of the infection is the same, colonization of the stomach, and the organisms are the same.

The clinical study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with Good Clinical Practices(5) (GCP) and with the applicable regulatory requirements. The clinical protocol will be reviewed and approved by the Institutional Review Board/Ethical Committee (IRB/EC) at each participating institution or by a central IRB/EC.

4.1. Helicobacter pylori (H. pylori) Infections in Pediatrics

Exact estimates of the incidence and prevalence rates of *H. pylori* infection in children less than 18 years of age in the USA are not known at this time. There is evidence that early childhood is the critical period of *Helicobacter pylori* infection, since clustering of the

[2] http://emedicine.medscape.com/article/929452-overview

infection within families suggests a major role of interfamilial transmission[6]. However, it is well known that the prevalence of peptic ulcer disease in childhood is extremely low. Generally, if there is a suspicion of a duodenal ulceration, an esophagogastroduodenoscopy (EGD) is performed to confirm the existence of this condition. Large pediatric endoscopy centers report an incidence of 5 to 7 children with peptic ulcer disease per year[7]. Since almost all peptic ulcers in children are located in the duodenum, it is safe to conclude that the majority of gastric ulcers (which are extremely rare in children) are usually secondary to drugs, Crohn's disease, cystic fibrosis, etc. Due to the low prevalence of gastric ulcers in children, no significant studies have been done to evaluate any possible association between gastric ulcers and *H. pylori* infection in children.

However, a strong correlation has been shown between duodenal ulceration and *H. pylori* gastritis in children[8]. In fact, *H. pylori* gastritis has been found in 90% to 100% of pediatric subjects with duodenal ulcer disease[9]. Thus, finding a duodenal ulcer in children in the absence of H. pylori gastritis is extremely rare.

Although the gold standard for *H. pylori* testing is EGD and biopsy, the most accepted method of testing for *H .pylori* infection in the pediatric population is stool antigen testing. Breath testing is also common, but requires specialized laboratories for analysis and active breathing into breath collection bags is not always possible in young children.

In this study, breath testing with a nasal cannula will be performed for all children. This method (use of nasal cannula as part of ***IDkit:Hp™ One***) has been used in previous pediatric studies with the Exalenz BreathID device[3, 10, 11] and will be further confirmed in this study. Those children who can follow the instructions and breathe effectively into breath collection bags will also be tested using the ***IDkit:Hp™ Two*** breath collection bags.

4.2. Comparator Details

Due to the fact that esophagogastroduodenoscopy (EGD) is extremely uncommon in pediatrics, the comparator used in this trial is a standard test method used to diagnose *H. pylori* infection in pediatrics; that is: stool antigen testing as assessed by a central pathology laboratory. Fecal antigen tests detect antigens in stool samples. Enzyme-linked immunosorbent assay (ELISA) formats comprising monoclonal antibodies against *H. pylori* proteins showed improved results compared to polyclonal approaches[12]. The current guideline evaluates the use of the stool antigen test as equivalent to the UBT if a validated laboratory-based monoclonal antibody is used [13]. Korkmaz *et al.* [14] compared the diagnostic accuracy of five different stool antigen tests in adult dyspeptic subjects

comparing monoclonal enzyme immunoassay tests (EIA) and rapid immunochromatographic assays (ICA). The sensitivity and specificity of the tests analyzed had a high variation between 48.9%–92.2% and 88.9%–94.4%, respectively, depending on the test format. Best results were obtained for *Premier Platinum HpSA Plus* (which will be used in this study) with sensitivity and specificity of 92.2% and 94.4%, respectively. They conclude that EIA tests are more accurate compared to the currently available ICA-based test, that are fast and easy to use but provide less reliable results[14]. A recent meta-analysis conducted by Zhou and colleagues analyzed forty-five studies, including 5931 subjects, and evaluated the test performance of a *H. pylori* stool antigen test in children. The average sensitivity and specificity was 92.1% and 94.1%, respectively[15]. In a study conducted in South Korea on 515 subjects comparing the stool antigen test to histology, RUT, ^{13}C-Urea breath test and serology, the accuracy of the stool antigen test ranged from 93% to 96%[16]. Furthermore, the available stool antigen tests have been shown to be able to distinguish infected from treated subjects[17, 18], enabling the confirmation of treatment. Degradation of antigens in the intestine and consequent disintegration of epitopes might lead to false negative results. Moreover, the process of sample handling could be difficult for subjects. False negative results may occur when the bacterial load is low, due to proton-pump inhibitors or the recent use of antibiotics or bismuth[19, 20]. As such, there are limitations in eligibility criteria and study procedures in order to reduce the possibility of any potential effect by these factors[10].

4.3. The ^{13}C-Urea Breath Test

The ^{13}C-Urea Breath Test (UBT) is a non-invasive test for detecting the presence of *Helicobacter pylori* (*H. pylori*) infection by the organism's urease activity. In the presence of *H. pylori*, the ingestion of urea, labeled with the non-radioactive isotope ^{13}C, results in production of labeled $^{13}CO_2$, which can be quantified in exhaled breath.

A solution containing ^{13}C-urea is given orally. ^{13}C (read as 'carbon 13') is a stable, non-radioactive isotope. In the presence of *H. pylori* infection, bacterial urease splits the urea to produce $^{13}CO_2$ and ammonia. The $^{13}CO_2$ diffuses rapidly into the bloodstream and is excreted by the lungs. The increase of $^{13}CO_2$ in breath can be measured and detected by specific analyzers (*e.g.,* the *BreathID® Hp* device).

Because the normal human stomach is devoid of urease, any gastric urease activity is considered to be derived from *H. pylori*. Therefore, demonstration of urease activity in the stomach is a reliable surrogate marker of active *H. pylori* infection[21]. The test aims at

giving reliable results without need for an invasive procedure. UBTs have high accuracy and reproducibility because they are functional tests that essentially "sample" the entire stomach; they are not prone to the same level of sampling error as biopsy-based tests may be. False positive results are uncommon with UBTs[22]. The sensitivity and specificity of the breath test ranges from 90% to 100%, while in most cases it is above 95%[21, 23-26]. Upper esophagogastroduodenal (EGD) endoscopy is considered the reference (or gold standard) method for the diagnosis of *H. pylori* infection, as well as UBT[4, 27, 28]. For the pediatric population, EGD is usually avoided and breath tests or stool antigen tests are generally used. Although not pleasant, stool antigen testing is widely used due to the ease and availability of the test and its relatively high accuracy (approximately 95%).

4.4. Breath Analyzing Devices and Breath Collection Methods

Metabolism of the labeled compound (^{13}C-urea) is assessed by measuring the ratio of $^{13}CO_2/^{12}CO_2$ in exhaled breath. In order to assess this ratio the breath needs to be collected before ingestion of the labeled substrate and after ingestion. Continuous breath collection utilizes a nasal cannula connected to a measuring device and is cleared for use in adults based on Premarket Notification (K011668, K130524). This method can be especially useful in children as the device automatically collects the breath via nasal cannula and does not require the child blow forcefully into bags or tubes. When using breath collection bags, the two breath samples are collected and then analyzed later by the ***BreathID® Hp Lab System***. Systems using this method have been already cleared by the FDA (e.g. K974322, K000316, K013371). The method of using breath sample bags in the diagnosis of *H. pylori* is common in the US and in other countries [23], but it requires more subject cooperation in order to fill the breath collection bags with adequate breath samples, which can be difficult for young children.

4.4.1. Exalenz' BreathID® Hp System (K130524)

Exalenz' established and marketed ***BreathID® Hp System*** (K130524) includes the *BreathID® Hp* device, an *in vitro* diagnostic (IVD) Class I (product code MSQ, Microbiology) which is used with the test kit ***IDkit:Hp™ One*** cleared in adults.

The *BreathID® Hp* device is a modification of the ***BreathID® System*** that was originally cleared by the FDA in July 2001 (K011668) and utilizes the same test kit, ***IDkit:Hp™ One***. This portable device senses CO_2 in exhaled breath, and analyzes its different isotopes in real-time based on specific optical-radiation emission and absorption by $^{13}CO_2$ and $^{12}CO_2$ gases. The device calculates the change in the $^{13}CO_2/^{12}CO_2$ ratio from exhaled breath[1]

before and after ingestion of ^{13}C labeled urea and produces a Delta-over-Baseline (DOB[3]) value that is printed out on an automatic printout. The procedure uses a nasal cannula that samples the subject's breath continually and provides an immediate answer within 10 – 30 minutes for the overall procedure including the ingestion of the ^{13}C labeled substrate.

Over 150,000 subjects have been assessed worldwide using the ***BreathID®*** and ***BreathID® Hp Systems*** with its test kit - ***IDkit:Hp™ One***.

Three studies were published using the BreathID device with nasal cannula and 75mg ^{13}C-urea in over 100 pediatric subjects[(2, 10, 11)]. Furthermore, a similar product has been approved for marketing in the pediatric population (PMA# P1000254). As such and as confirmed with FDA (Q170072), the UBT with the data obtained for adults can be extrapolated for pediatrics and a confirmatory trial is deemed sufficient.

4.4.2. Exalenz' BreathID® Hp Lab System (K162150)

Exalenz' recently extended the panel of breath collection methods by establishing and obtaining clearance for marketing of the ***BreathID® Hp Lab System*** (K162150) to satisfy marketing requirements and enable the use of Exalenz ^{13}C-Urea Breath Test with low capital costs. The ***BreathID® Hp System*** (K130524) was modified to enable breath testing and the calculations based on two breath sample bags collected at specified times, instead of using the nasal cannula, thereby enabling off site testing when a device is not available for online measurements. The test kit, ***IDkit:Hp™ Two***, contains the same ^{13}C-urea/citric acid reagent (NDA-21-314) as in the original ***IDkit:Hp™ One***, but instead of a nasal cannula it contains two breath sample bags. The bags are specially designed to store the exhaled breath samples until analysis for up to 14 days from the time of collection.

The analyzer unit, the ***BreathID® Hp*** device, was modified with software enabling the bag measurements. Together with the ***IDkit:Hp™ Two,*** an ***Auto Sampler***, and the ***BreathID® Hp Lab Application*** installed on a standard computer, these components form the ***BreathID® Hp Lab System***. The ***BreathID® Hp Lab System*** allows consecutive and automatic analysis of the ***IDkit:Hp™ Two*** breath collection bags. The first sample, collected immediately before ingesting the ^{13}C-urea/citric acid solution, defines the baseline ratio of

[3] Delta Over Baseline (DOB) is defined as: $[(^{13}CO_2^{(n)}/\,^{12}CO_2^{(n)} - {}^{13}CO_2^{(0)}/\,^{12}CO_2^{(0)})*1000]/\,(^{13}CO_2^{(PDB)}/\,^{12}CO_2^{(PDB)})$, where PDB is the standard ^{13}C/^{12}C isotope ratio (=1.1273%); (0) is the base line measurement and (n) is the measurement of interest. DOB ≥ 5 indicate *H. pylori* infection

$^{13}CO_2/^{12}CO_2$. The second sample, collected 15-20 minutes following the ^{13}C-urea/citric acid solution ingestion, is analyzed to determine changes in the $^{13}CO_2/^{12}CO_2$ ratio.

4.5. Rationale of performing UBT with two methods of breath collection

Although ^{13}C-urea breath tests have been in use for over three decades, most testing has been with breath collection bags or test tubes. The procedure using the ***BreathID®*** and ***BreathID® Hp Systems*** with a nasal cannula (***IDkit:Hp™ One)*** where the subject's breath is continually collected has the advantage of providing an immediate answer within 10 – 20 minutes. This is also advantageous in children who have difficulty exhaling actively into breath collection bags. To facilitate testing in clinics that cannot accommodate a ***BreathID® Hp System*** on site, and allow higher volumes of testing, Exalenz has developed an additional test kit, the ***IDkit:Hp™ Two,*** that incorporates the same ^{13}C-labeled urea tablet and citric powder (approved under NDA-21-314) as ***IDkit:Hp™ One***, but instead of using a nasal cannula for breath collection, a pair of breath sample bags are used. The bags are specially designed to store the exhaled breath samples until analysis for up to 14 days from the time of collection.

The pair of filled breath sample bags will be analyzed using the ***BreathID® Hp Lab System***.

5. INTENDED USE *BREATHID® HP SYSTEM*

The Exalenz ***BreathID® Hp System*** is intended for use to continually and non-invasively measure changes in the $^{13}CO_2/^{12}CO_2$ ratio of exhaled breath, which may be indicative of increased urease production associated with active *Helicobacter pylori* (*H. pylori*) infection in the stomach. The Exalenz ***BreathID® Hp System*** is indicated for use as an aid in the initial diagnosis and post treatment monitoring of *H. pylori* infection in adult and pediatric patients. The Exalenz ***BreathID® Hp System*** consists of the ***IDkit: Hp™ One*** and the ***BreathID® Hp*** test device.

The device is for use by trained health care professionals. To be administered under a physician's supervision.

6. INTENDED USE *BREATHID® HP LAB SYSTEM*

The Exalenz ***BreathID® Hp Lab System*** is intended for use to non-invasively measure changes in the $^{13}CO_2/^{12}CO_2$ ratio of exhaled breath, which may be indicative of increased urease production associated with active *H. pylori* infection in the stomach. The Exalenz ***BreathID® Hp Lab System*** is indicated for use as an aid in the initial diagnosis and post treatment monitoring of *H. pylori* infection in adult and pediatric patients. The Exalenz ***BreathID® Hp Lab System*** consists of the ***IDkit: Hp™ Two*** kits, and the ***BreathID® Hp*** device, ***Auto Sampler*** and ***Lab Application***.

The device is suitable for use in both point of care and clinical laboratory settings. To be administered by trained personnel as ordered by a licensed healthcare practitioner.

7. BREATHID® HP SYSTEM – DESCRIPTION

The ***BreathID® Hp System*** is a stand-alone table top device for real-time breath testing. The device includes custom imbedded software specifically for use with the intended application. The user screen provides self-explanatory messages that lead the user through the test procedure. The operation is via a touch screen and the subject is connected to the device with a nasal cannula. This device provides a printout with the final DOB result automatically when the test is completed after a maximum of 20 minutes. A detailed description of the device and its operation appear in the Operator Manual.

Figure 1: The *BreathID® Hp* System

The device elements are summarized in the following table:

Element	Description
Device Display	The device display is located on the front panel and is the user interface of the BreathID® Hp. All BreathID® Hp controls, instructions, and messages appear on this screen. Controls may be selected by touching the screen.
IDcircuit/Input Connector	The IDcircuit or Bag Adaptor is connected here. The IDcircuit/Input Connector has a protective cover that covers the breath entry port when the BreathID® Hp is not in use.
Printer	The printer is located to the right of the screen.
BreathID® Hp LED Indicator	There is a Green indicator LED located on the front panel of the BreathID® Hp.
USB Port	The USB port is located on the front panel (above the screen) and is used for connecting the Exalenz **Flash Drive** to the BreathID® Hp.
Power ON/OFF Switch	This switch is located on the back of the device.
AC Connector	The AC Connector is located on the back panel of the BreathID® Hp. The AC power cord is attached to this connector.
Printer LED Indicators	There are two indicator LEDs located on the printer.
Printer Controls	There are two controls located on the printer. One is used to advance the paper feed, and one is used to cut the printer paper.
Printer Open Button	Pressing this button opens the printer compartment cover.

The ***BreathID® Hp System*** is used with the ***IDkit:Hp™ One***. The following materials are included in this kit:

- One 75 mg tablet ^{13}C-enriched urea
- One packet of granulated Citrica (4 gram of artificially sweetened citric acid powder)
- One stirring and drinking straw
- One Package insert for the breath test
- IDcircuit (nasal cannula)

Required materials not provided:

- Drinking cup
- Tap water

8. BREATHID® HP LAB SYSTEM TEST – DESCRIPTION AND COMPONENTS

The ***BreathID® Hp Lab System (P/N VS01082)*** is suitable for Clinical Laboratory settings. It is to be administered by trained personnel as ordered by a licensed healthcare practitioner.

As previously mentioned, the ***BreathID® Hp Lab System*** consists of the ***IDkit:Hp™ Two*** kits and the ***BreathID® Hp Lab*** device, ***Auto Sampler*** and ***Lab Application***. The components of the ***BreathID® Hp Lab System*** are described below:

1. ***BreathID® Hp Lab*** **device** - a table top device weighing approximately 33 lbs (15kg) (Figure 1). The user screen on the device is inactive, and the user interface is on the screen of the computer on which the ***BreathID® Hp Lab Application*** is installed. The device includes custom embedded software specifically for use with the intended application.

   Figure 2: The *BreathID® Hp Lab* device

2. ***Auto Sampler*** - a free standing module connected to the device with 20 ports for loading up to 10 sets of breath collection bags (Figure 3). The breath sample transfer to the ***BreathID® Hp*** device is controlled and synchronized by the ***Lab Application***.

Figure 3: The *BreathID® Hp* - Auto Sampler

3. ***BreathID® Hp Lab Application/Work station -*** a personal computer that contains specialized software that controls the entire system and provides a touch - sensitive user screen for the operator. The ***Lab Application*** controls and synchronizes between the ***Auto Sampler*** and the ***BreathID® Hp Lab device.*** The ***Lab Application*** also instructs the user on performing bag tests and displays the results obtained from the device.
4. ***IDkit:Hp™ Two breath sample bags*** - The entire list of components of this kit will be described in the next section. The breath sample bags are part of the ***IDkit:Hp™ Two*** and are used to collect the subject's breath at baseline, before ingestion of the test drink, and 15 minutes (and no more than 20 minutes) post ingestion. They can be measured at a later time up to 14 days following collection and at any location with the equipment described above.

Figure 4: *IDkit:Hp™ Two* breath sample bags

5. **Components of *IDkit:Hp™ Two***

The ***IDkit:Hp™ Two*** kit components:

a. Two breath sample bags[4] (baseline and post ingestion)

b. One 75 mg tablet ^{13}Cenriched urea

c. One packet of granulated Citrica (4 gram of artificially sweetened citric acid powder)

d. One stirring/ drinking straw

e. One large sample transport bag provided to store/ship both breath sample bags

f. One *Quick User Guide* showing the basic steps of administering the test

Figure 5: Components of the *IDkit:Hp™ Two*

Required materials not provided:

- Drinking cup
- Tap water
- Timer

Note: In this study, ***IDkit:Hp™ Two*** *will be used, with the addition of a nasal cannula (part of* ***IDkit:Hp One****).*

9. STUDY DESIGN

Multi-center, non-randomized, open label study, designed to confirm the safety and the efficacy of ^{13}C-urea breath test using the ***BreathID® Hp*** and the ***BreathID® Hp Lab Systems*** in pediatric subjects and confirm the efficacy of the respective means of breath collection, the nasal cannula and breath collection bags, in this population compared to *stool antigen* testing. A central lab will analyze the stool specimens. Children, that cannot inflate the breath collection bags, may perform UBT only with nasal cannula. All children will be exposed to non-radioactive ^{13}C-urea and citric acid.

Subjects will be enrolled on a walk-in basis. Subjects will be treated only based on the established methods used in clinical practice. No patient management decisions should be made based on the investigational ***BreathID® Hp*** and/or ***BreathID® Hp Lab Systems*** test outcomes and the investigator and treating physician will remain blinded to the ***BreathID® Hp*** and/or ***BreathID® Hp Lab System*** test results until the end of the study.

Appropriate measures will be taken to maintain blinding.

A minimum of 40 evaluable, with at least 5 infected, subjects will be accrued. If the minimum of 5 positive subjects is not reached within the first 40 subjects enrolled, enrollment will continue until this minimal number of positive subjects is reached (with evaluable UBT results for both breath collection methods). The actual number of recruited subjects may be slightly higher due to parallel recruiting in multiple sites. The main eligibility criteria for the trial are subjects that have a clinical indication for *H. pylori* testing and are naïve to *H. pylori* treatment in the past 6 weeks.

10. BREATH TESTING PROCEDURE

10.1. Collecting the Breath Samples

Prior to filling the bags, complete all fields including the subject number and initials (except for filling times that will be written immediately after filling the bags) on the bag identification label (blue BASELINE and gray POST INGESTION breath bags) and write subject's number and initials on both bags.

All subjects will undergo the breath test using the ***IDkit:Hp One*** nasal cannula. The unmasked study personnel will perform this test and the print out will be kept in a separate binder with no access for the investigator and/or treating physician. Subjects that can and are

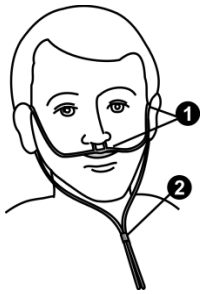
willing to exhale into ***IDkit:Hp™ Two*** breath collection bags, will breathe into them in parallel as described below.

I. Bag filling of baseline breath sample *(only for those subject able to fill the breath collection bags)***:**

Ask the subject to take a deep breath, wait 4 - 5 seconds and then exhale into the blue *BASELINE* breath bag. Ascertain that the cap of the bag is returned securely into place until it clicks, after filling the breath collection bag.

II. Beginning test on the *BreathID® Hp System*:

Connect the IDcircuit (nasal cannula) using the following procedure:



1. Slide the tubing sleeve down and place the cannula tips gently into the subject's nostrils, with the lip guards placed on the upper lip. Place the cannula tubing over the ears.
2. Slide the tubing sleeve up toward the neck to fit comfortably under the chin.
3. Twist one orange connector clockwise securely into the device as shown.
4. Verify that the IDcircuit is not twisted or kinked, and that the cannula tips are in the nostrils.

Note: Subject should breathe normally throughout the test and refrain from talking.

5. Press the Start button on the device to proceed and measure baseline. This will usually take 2 minutes but may take up to 12 minutes when calibration is necessary.

III. Preparation and Administration of the Test Drink

Note: Administer the test drink within two hours of preparation, as this is the maximal time for maintaining solution stability.

1. Empty the contents of the packets with Citrica and with ^{13}C-enriched urea tablet in 150 to 200 ml (5.1 to 6.8 oz) of tap water in a single drinking cup of at least 236 ml (8 oz.) in capacity.
2. Stir thoroughly with the provided straw for a few minutes until the Citrica powder and the ^{13}C-urea tablet are completely dissolved. Keep the stirring straw in the cup to serve for drinking.

Note: Tiny particles may remain visible after thorough mixing. However, if more substantial particulate matter is still present after five minutes, discard the solution and repeat the procedure with a new kit.

3. Administer solution to the subject when Citrica powder and urea tablet are completely dissolved and begin measuring 15 minutes.
4. Record the time of ingestion on the blue *BASELINE* breath bag.

IV. Collecting Breath samples after ingestion of the test substrate

1. Immediately after the subject finishes drinking the solution, press the Start button on the ***BreathID® Hp System*** screen to proceed.
2. At least 15 minutes after the administration of the test drink (but not later than 20 minutes after administration), ask the subject to take a deep breath, wait 4-5 seconds and then exhale into the gray *POST INGESTION* breath bag. Ascertain that the cap of the bag is returned securely into place until it clicks.
3. Record the actual time filled on the gray breath *POST INGESTION* bag.
4. Once the ***BreathID® Hp System*** has completed the measurement it will prompt the user to disconnect the cannula. Please remove it from the subject's nostrils and twist the orange connector counterclockwise removing it from the device. The used cannula should be discarded according to local operating procedures for disposal of medical waste.

V. Storage of Bags for future measurement

Assure that all fields are complete on the breath sample bag labels, for future identification. The bags should be stored until they are tested at room temperature in a safe place where they will not get destroyed or be exposed to high pressure.

10.2. Measuring Bags

The bags will be measured by the ***BreathID® Hp Lab System*** whose operators will be trained prior to performing measurements of the bags by authorized Exalenz personnel according to the ***BreathID® Hp Lab System Operator Manual***.

The bags should be measured within 14 days.

After testing, although the bags were not designed for re-testing, all bags should be stored under the same conditions for potential collection by the sponsor for research purposes. When instructed by the Sponsor in writing, they may be discarded.

11. STUDY OBJECTIVES

This study is primarily designed to confirm tolerance of the dose used for the pediatric population. The ^{13}C-urea and citric acid (NDA-21-314) are contained in ***IDkit:Hp™ One*** in the ***BreathID® Hp System*** (K130524) and ***IDkit:Hp™ Two*** in the ***BreathID® Hp Lab System*** (K162150). Additionally the efficacy of the respective means of breath collection, the nasal cannula contained in ***IDkit:Hp™ One*** and breath collection bags contained in ***IDkit:Hp™ Two***, will be confirmed to be similar in the pediatric population as that of adults.

Three studies were published using the BreathID device with nasal cannula and 75mg ^{13}C-urea in over 100 pediatric subjects[2, 10, 11]. As such, the UBT with the data obtained for adults can be extrapolated for pediatrics (as confirmed with FDA through Q170072) and a confirmatory trial is deemed sufficient.

11.1. Primary Objective: Safety

The safety of the ^{13}C-urea/citric acid solution has been previously established in adults and in children in literature.[1-3]Any adverse event (whether related or not) will be recorded. There are no safety concerns and no adverse events anticipated for the breath test or the stool antigen test beyond those already listed in the respective labels..

11.2. Secondary Objectives: Confirmation of Efficacy

1. To confirm overall agreement measures with lower 95% confidence limit ≥75% for the ***BreathID® Hp System*** where breath is collected via a ***IDkit:Hp™ One***

nasal cannula using the 5 DOB threshold in pediatric subjects (presence/absence of *H. pylori*) compared to stool antigen testing.

2. To confirm overall agreement measures with lower 95% confidence limit > 75% for the ***BreathID® Hp Lab System*** when analyzing ***IDkit:Hp™ Two*** breath sample bags using the 5 DOB threshold in pediatric subjects (presence/absence of *H. pylori*) compared to stool antigen testing.

12. SUBJECT SELECTION

Consecutive pediatric subjects aged between 3 and 18 years of age evaluated by the physician, or presented by referral from other institutions or physicians who are clinically indicated to undergo *H. pylori* testing and meet study criteria will be enrolled. Distribution of subject's ages will be monitored to assure all age ranges are represented. To confirm safety and performance of the tests in younger children, this study will predominantly (approximately 2/3) enroll children under the age of 12 to confirm drug/dose tolerance and safety and confirm its efficacy.

Consent to participate in the study and to undergo the breath test procedures must be obtained from the legal representative/guardian of every subject following a thorough explanation of the study, including the benefits and risks (if any). The majority (approximately 2/3) of the subjects will be from US centers. Assent will also be obtained from those subjects above the age that local regulations require consenting of the child.

All subjects screened will be documented in a screening log, regardless of subsequent entry into the study. Reasons for non-inclusion will be recorded.

12.1. Inclusion Criteria

To be eligible to participate in this study, subjects must meet all of the following inclusion criteria:

- Be older than 3 and younger than 18 years of age
- Present with clinical indication compatible with *H. pylori* based on the judgement of the treating physician (such as abdominal pain, nausea, diarrhea, reflux, peptic ulcer, dyspepsia, etc., or following treatment for *H.pylori*)
- Subject/Legal guardian (and subject when relevant) is willing to sign the Informed Consent/Assent Form

- Naïve to *H. pylori* treatment in the past 6 weeks

12.2. Exclusion Criteria

To be eligible to participate in this study, subjects must not meet any of the following exclusion criteria:

- Participation in other interventional trials
- PPI or H_2 blockers within two (2) weeks prior to breath test/stool antigen test
- Pregnant or breastfeeding female
- Allergy to test substrates
- Antibiotics (not related to *H. pylori* eradication) and/or Bismuth preparations within four (4) weeks prior to breath test
- Exposure to any type of anesthesia, analgesics or sedation 24 hours prior to the breath test.
- Exposure to any ^{13}C-enriched substance 24 hours prior to the breath test.
- Children 12 years and older – to be excluded after a written notification from the sponsor is received at the site that the limit of 1/3 of the sample size was achieved for this group
- Subjects outside US – to be excluded after a written notification from the sponsor is received at the site that the limit of 1/3 of the sample size was achieved for this group

12.3. Exclusion Criteria for Day of Urea Breath Test

- Subject did not fast for the hour prior to the UBT

12.4. Consenting

Subject's legal guardian(s) will sign a consent form prior to study participation.

Subjects, old enough according to local regulations, to understand and sign an assent will do so prior to study participation.

The consent/assent will include willingness to share pertinent blood, clinical and imaging data that appear in the subject's health record and incorporate all data from time of enrollment until the subject's termination of the study.

12.5. Termination of Subjects

The study subject may be terminated in an individual case by the subject or his/her legal guardian if for some reason after giving written consent/assent, the subject or his/her legal guardian wishes to withdraw and/or discontinue his/her participation in the study. This will not have any effect on the treatment that he/she receives.

12.6. Early Withdrawal from the Study

Subjects, their relatives, their representatives or the subjects' physician may withdraw the subject from the study at any time if they feel the study is not in the subjects' best interest, or they may be withdrawn by the investigator or sponsor for safety, compliance issues, or administrative reasons. All withdrawals will be fully documented as to time and cause.

If the subject or his/her legal guardian withdraws from the study and also withdraws consent for the disclosure of future information, no further evaluations should be performed and no additional data should be collected. This will not have any effect on the treatment that the subject receives. The subject's data that have been collected until the time of withdrawal will be retained and analyzed.

12.7. Expected Duration of Recruitment

The study is planned for a duration of approximately 6 months.

13. STUDY ASSESSMENTS AND PROCEDURES

13.1. Study Visits

The clinical study will be conducted in compliance with this protocol and in accordance with the ethical principles under Investigational Review Board (IRB) or Helsinki committee approval consistent with Good Clinical Practice (GCP) and with the applicable local regulatory requirements.

The investigator(s) at each site will sign a Protocol Approval Page (**Appendix A**).

Screening and Baseline can be performed at the same visit

13.1.1. Screening Visit (can be done up to 37 days prior to the breath test)

1. The legal guardian (and subject if applicable) will sign the consent/assent form prior to performing any study related procedures. The child will sign the assent form if, based on his age, local regulations require the child to sign the form.
2. The investigator(s), co-investigator(s) or study coordinator will record:
    - Demographic information.
    - Medical history including information regarding H. pylori results.
    - Concomitant medications (starting 6 weeks prior to the planned breath test/stool test)

    This will be performed for each subject in order to establish compliance with the inclusion and exclusion criteria.

13.1.2. Baseline Visit Day 1

1. The investigator(s), co-investigator(s) or study coordinator will record any changes from screening visit in medical history including changes in concomitant medications (starting 6 weeks prior to the breath test/stool test) for each subject to establish compliance with the inclusion and exclusion criteria.
2. Physical examination including height and weight of the subject will be performed.
3. A urine pregnancy test will be performed for females of childbearing potential who did not have a pregnancy test within 7 days prior to the breath test.
4. Inclusion and exclusion criteria will be checked.
5. Only once the subject's eligibility has been confirmed, and after refraining at least 2 weeks from proton pump inhibitors and 4 weeks from antimicrobials and bismuth preparations, the subjects will perform the UBT (according to the procedure described in Section 10 above) by filling two ***IDkit:Hp™ Two*** breath collection bags, one at each time point (pre-ingestion and 15-20 minutes post-test solution ingestion) and by having breath collected via the ***IDkit:Hp™ One*** nasal cannula. Subjects unable to fill the breath collection bags will perform the breath test with the nasal cannula only.

6. Adverse events will be recorded, accounting for the rules of assessment and documentation (see Section 17 below).
7. Breath collection bags will be analyzed by the ***BreathID® Hp Lab System*** within 14 days of collection either on site or at a central laboratory. When relevant, the site coordinator will arrange shipment of the breath collection bags to a central laboratory, including a completed requisition form. The name and the address for the central laboratory service provider can be found in the respective documentation.
8. The investigator and treating physician will remain blinded until the end of the study and patient management will not be affected by the breath test results.

This visit can be combined with screening visit and performed on the same day.

13.1.3. Stool sample collection visit (Day 1 +/- 7 days)

1. The stool antigen test will be performed within 7 days before or after the UBT and after at least 2 weeks of refraining from proton pump inhibitors & H2 blockers and 4 weeks of refraining from antimicrobials and bismuth preparations. The test procedure is described in Appendix B.

2. Stool specimens will be sent to a central lab. The site coordinator will provide each subject with all supplies needed for collection of the stool specimen. The specimen will be either collected on-site, brought back to the site, brought to a local approved stool collection facility or it may be sent directly to a central laboratory from the subject's residence. A requisition form will be completed. After confirming that each stool antigen sample was sent to the central lab, the study coordinator will complete an entry line in a log documenting the shipment. The name and the address for the central laboratory service provider can be found in the respective documentation

3. Adverse events will be recorded, according to rules of assessment and documentation defined in Section 17 below).

   The visit can be combined with the Breath Test visit and/or with the Screening visit and performed on the same day.

For each subject tested, an individual set of electronic Case Report Forms (CRFs) will be completed.

The Sponsor will notify the sites when to commence and stop recruitment.

13.1. Schedule of activities and evaluations

Visit	Screening	Baseline Day 1	Stool Testing
Day	**Up to 37 days prior to the breath test**	**0-7**	**UBT +/- 7 days**
Informed consent Including Assent if applicable	X		
Inclusion & exclusion criteria	X	X	X
Demographic information	X		
Medical history information regarding previous *H. pylori* results	X	X	
Prior and concomitant medications (starting 6 weeks prior to the Stool Test/UBT)	X	X	X
Physical examination including height and weight		X	
Urine pregnancy test		X	
Adverse event assessment		X	X
UBT - ^{13}C-Urea Breath Test (cannula & bags)		X	
Stool Testing			X

Note:
Screening visit can be combined with Day 1 visit and performed on the same day.

Stool test visit can be combined with Day 1 visit or screening visit and performed on the same day.

Breath collection bags will be tested within 14 days after UBT visit.

13.2. Screen failure Re-screening and dropout

Every subject for whom an Inform Consent/Assent Form was signed and withdrew from the study for any reason prior to the UBT or stool test will be defined as a *Screen Failure*.

Re-screening will be allowed once for each subject.

Every subject for whom an Inform Consent/Assent Form was signed and withdrew from the study for any reason after UBT and/or stool testing started will be defined as dropout/early termination. The cause of each such case will be recorded.

13.3. Study assessments

13.3.1. Stool Test

The stool specimens will be sent to a central lab. The site coordinator will provide each subject with all supplies needed for collection. The specimen will be either collected on-site, brought back to the site, brought to a local approved stool collection facility or it may be sent directly to a central laboratory from the subject's residence. A requisition form will be completed. After confirming that each stool antigen sample was sent to the central lab, the study coordinator will complete an entry line in a log documenting the shipment. The central lab will proceed in the analysis of the specimen according to the stool test manufacturer's instructions.

13.3.2. Breath Test

The results from the ***BreathID® Hp System*** will be printed out immediately after the test and will serve as source data for the CRF completion. Appropriate measures will be taken to maintain blinding of the investigator and treating physician to the breath test results. The breath test collection bags will be measured within 14 days of collection using the ***BreathID® Hp Lab System*** according to the respective User Manual.

13.4. Investigational Product Handling

The Investigator and/or Research Pharmacist (as appropriate per site) will be provided with guidance regarding the packaging and labeling requirements, receipt of investigational product, dispensing and accountability procedures, preparation instructions, storage and stability of Investigational product, Disposition of Investigational Product, with the following forms required: Proof of receipt form, Temperature logs, Accountability logs, Investigational product and material transfer/disposition form and pharmacy staff identification log (if applicable).

The kits containing the substrate should be stored at room temperature (15-30°C, 59-86°F), in a restricted area or cabinet, and will be inaccessible to unauthorized personnel. The sponsor should be notified regarding any temperature excursion on site, and the substrate

should be kept in quarantine until a written approval from the sponsor that the substrate can be used has been received.

Local forms may be authorized for use after being approved by the Sponsor or his assigned representative.

13.5. Investigational Product Accountability

The Investigator or their designee, are responsible for ensuring that all study supplies received at the site are inventoried and accounted for throughout the study. The dispensing of study test kits for the individual subject must be documented in the respective accountability form. The study Investigational Product must be handled in strict accordance with the protocol, GCP and GMP requirements, and the container label and will be stored in a limited access area or in a locked cabinet under appropriate environmental conditions. Unused study materials must be available for verification by the sponsor's site monitor during on-site monitoring. Used packages of the substrate should also be kept for accountability purposes and will be destroyed at the site only after accountability is completed by the sponsor. The destruction of unused study materials (both, expired or unexpired) will be documented on the return/disposition form. The Sponsor will authorize destruction of excess supplies on site according to local policy. In this case, before proceeding, the site must seek written authorization from the Sponsor using the return/destruction form and this must also be documented on the Study Supply Return Form.

Study test solutions should be dispensed under the supervision of the investigator, a qualified member of the investigational staff, or by the hospital clinical pharmacist.

14. STATISTICAL CONSIDERATIONS

14.1. Study Design and Objectives

The study is designed as multi-center, open label study, designed to confirm the safety and the efficacy of ^{13}C-urea breath test using the ***BreathID® Hp and BreathID® Hp Lab Systems*** in pediatric subjects and confirm the efficacy of the respective means of breath collection, the nasal cannula and breath collection bags, in this population compared to *stool antigen* testing. A central lab will analyze the stool specimens. Children, that cannot inflate the breath collection bags, may perform UBT only with ***IDkit:Hp One*** nasal cannula. All children will be exposed to non-radioactive ^{13}C-urea and citric acid. Subjects will be enrolled on a walk-in basis. Subjects will be treated only based on the established methods

used in the local routine clinical practice and the investigator and treating physician will remain blinded to the breath test results until the end of the study.

14.2. Endpoints

14.2.1. Primary Endpoint: Safety

Adverse event rate: The safety of the ^{13}C-urea/citric acid solution has been previously established in adults and in children in literature[1-3]. Any adverse event (whether related or not) will be recorded. There are no safety concerns and no adverse events anticipated for the breath tests.

14.2.2. Secondary Endpoints: Efficacy

1. Overall agreement in diagnosis (presence/absence of *H. pylori*) between the ***BreathID® Hp System*** results where breath is collected using ***IDkit:Hp™ One*** via a nasal cannula applying the 5 DOB threshold in pediatric subjects and stool antigen outcome or biopsy results in the pediatric population.
2. Overall agreement in diagnosis (presence/absence of *H. pylori*) between the ***BreathID® Hp Lab System*** results when analyzing ***IDkit:Hp™ Two*** breath sample bags using the 5 DOB threshold in pediatric subjects and the stool antigen outcome or biopsy results in the pediatric population.

14.2.3. Study Hypotheses

In this study, the following two sets of hypotheses will be evaluated for both the ***IDkit:Hp One*** used with the ***BreathID® Hp System*** and ***IDkit:Hp™ Two*** used with the ***BreathID® Hp Lab System***.

- H_0: Overall agreement < 75%
- H_1: Overall agreement ≥ 75%

14.3. Sample Size

The sample size for this study of pediatric subjects with indications for *H. pylori* testing is calculated to allow confirmation of both, the primary and secondary study endpoints. For the safety assessment, with no related SAE's expected, a sample size of 35 evaluable subjects would provide an upper confidence limit with less than or equal to 10% for the 95% two sided confidence limit. In order to support the efficacy endpoints a sample size is calculated through the width of the two-sided 95% confidence interval of the percent overall

agreement, such that the lower limit of the confidence interval will be greater than or equal to 75% with a point estimate of 90%.

A total of at least 40 evaluable subjects with no less than at least 5 positive subjects will be accrued for both breath collection methods. If the minimum of 5 positive subjects is not reached within the first 40 subjects enrolled, enrolment will continue until this minimal number of positive subjects is reached (with evaluable UBT results for both breath collection methods).

This will be sufficient to confirm the safety and the utility of the ***BreathID® Hp System*** and ***BreathID® Hp Lab System*** in detecting *H.pylori* infection in pediatric subjects.

Distribution of subject's ages will be monitored to assure all age ranges are represented, while predominantly (approximately 2/3) enrolling children under the age of 12.

14.4. Randomization

The study is an open label study, and is not randomized.

14.5. Analysis Sets

14.5.1. Full Analysis Set (FA)

The Full Analysis (FA) set includes all subjects who were enrolled and underwent study tests (***BreathID® Hp System*** when using ***IDkit:Hp™ One*** nasal cannula and/or ***BreathID® Hp Lab System*** when using ***IDkit:Hp™ Two*** breath sample bags and stool antigen testing).

14.5.2. Per Protocol Analysis Set (PP)

The Per-Protocol (PP) analysis set includes all subjects from the FA analysis set without any major protocol deviation.

14.6. Analysis set and their statistical analysis

The FA analysis set will serve as the analysis set for performance and safety assessments. The confirmatory performance assessment will also be performed on the PP analysis set.

14.7. Statistical Analysis

14.7.1. General Considerations

Statistical analyses will be performed using SAS® v9.4 (SAS Institute, Cary NC, USA).

If any statistical tests are performed, they will be two-sided. The required significance level of findings will be equal to or lower than 5%. Where confidence limits are appropriate, the confidence level will be 95%.

Baseline values are defined as the last valid value prior to study start.

All statistical analyses of safety and performance measures will be descriptive in nature. Continuous variables will be summarized by a mean, standard deviation, minimum, median and maximum, and categorical variables by a count and percentage. Confidence intervals will be provided where relevant.

If multiple measurements are taken in a single subject, statistics described below will be appropriately modified to accommodate the within subject correlation.

14.7.2. Demographic and Other Baseline Characteristics

Demographic, medical and clinical history variables will be tabulated. Continuous variables will be summarized by a mean, standard deviation, minimum, median and maximum, and categorical variables by a count and percentage.

14.7.3. Disposition of Subjects

The numbers of subjects who were enrolled will be provided, as well as the reasons for all enrollment discontinuations, grouped by major reason (e.g., lost to follow-up, adverse event, poor compliance). A list of discontinued subjects, protocol deviations, and subjects excluded from the efficacy analysis will be provided as well.

14.7.1. Safety Analysis

Any events related to study drug and/or device AE's rate will be presented along with a 95% exact binomial two sided 95% confidence interval. The analysis of all adverse events will include incidence tables and will include analyses by severity, relationship to device or drug and baseline variables.

14.7.2. Confirmatory Efficacy Analyses

A 2x2 table of the results comparing the positive/negative results obtained from the ***BreathID® Hp System*** and for the results of the ***BreathID® Hp Lab System*** applying the 5 DOB threshold and the stool antigen test results will be presented (each system compared separately to the stool antigen testing).

The confirmatory efficacy analysis will present the estimation of overall agreement (% on diagonal) with its two-sided 95% exact confidence intervals. If the lower limits of the two sided 95% confidence interval of the overall agreement are greater than or equal to 75%, the null-hypotheses will be rejected and we will have shown the breath test to be effective for the diagnosis of *H. pylori*. This will be performed for each breath collection method individually.

Positive and negative percent agreement will also be presented together with respective 95% confidence intervals.

Poolability of the data from the study sites will be assessed.

14.7.3. Handling of Missing Data

Measurements with unknown values or unavailable estimates will be treated as missing values and excluded from the analysis. No imputation of missing data will be performed.

15. ETHICS & REGULATORY CONSIDERATIONS

The study will be conducted in compliance with the principles of the Declaration of Helsinki (1996), the principles of GCP and in accordance with all applicable regulatory requirements including but not limited to the Code of Federal Regulations and FDA guidance documents.

This protocol and related documents will be submitted for review to all relevant IRBs or EC delegated to approve the study at their respective sites. Any amendment to the protocol may be proposed by the principal investigator. The amendment must be submitted to the relevant IRB or EC. When applicable, the implementation of the amendment will take place only once approved by the appropriate ethics committee.

Annual progress and safety reports and a final report at conclusion of the study will be submitted by Exalenz Bioscience (or on behalf of the Sponsor), to the FDA and the IRB/EC within the timelines defined in the Regulations.

16. INVESTIGATOR RESPONSIBILITIES

The investigator is responsible for ensuring that the clinical study is performed in accordance with the protocol, current ICH guidelines on GCP, US-GCP and applicable regulatory requirements. GCP is an international ethical and scientific quality standard for designing, conducting, recording, and reporting studies that involve the participation of human subjects. Compliance with this standard provides public assurance that the rights, safety, and well-being of study subjects are protected, consistent with the principles that originated in the Declaration of Helsinki, and that the clinical study data are credible.

The investigational devices and substrate accountability will be filed in the Investigator's Study File. Study supplies logged in will be kept by the investigator or the delegated persons in a secure place. All supplies (device, substrate / kits) will be used for this study only. After completion of the study, the device, kits and all unused accessories must be returned to Exalenz Bioscience Ltd. as per their request or alternatively, destroyed according to local regulations after receiving explicit authorization by Exalenz to do so and provide Exalenz with a confirmation.

The Principal Investigator will act as custodian for the study data. Subject data will be pseudonymized and it will be stored on password protected media. All data will be compliant with CFR and GCP.

17. SAFETY CONSIDERATIONS

17.1. Adverse Event Definitions

An adverse event (AE) is any undesirable or unintentional event that occurs in a patient or clinical investigation subject, whether or not considered related to the investigational product; this includes clinically significant changes in laboratory values. As this study is not therapeutic in nature, there will be no therapeutic failures to report.

Regardless of severity or relationship to the study investigational product, all diagnoses, symptom(s), sign(s) or finding(s) with a start date after the first study test has begun (Breath test/stool test) and until the last study procedure is being completed will be documented, assessed by the investigator and recorded in the subject's CRF.
A surgical procedure that was planned prior to the first study test by any physician treating the subject should not be recorded as an AE.

17.2. Serious Adverse Event (SAE), Unanticipated Adverse Device Effect (UADE) and Suspected Unexpected Serious Adverse Reactions (SUSAR)

A serious adverse event (SAE) is an event that is either:

a) Fatal
b) Life-threatening

Life threatening is defined as an event in which the patient is at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it was more severe.

c) Results in persistent or significant disability/incapacity

Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.

d) Requires or prolongs inpatient hospitalization
e) A congenital anomaly or birth defect.
f) Another serious or important medical event as judged by the investigator.

An Unanticipated Adverse Device Effect (UADE) or a Suspected Unexpected Serious Adverse Reactions (SUSAR) is an SAE, related to the investigational product, for which the nature or severity is not consistent with the expected outcomes of the treatment/testing being offered.

An unexpected adverse event is one that has not been previously observed, or one that is of a specificity or severity not consistent with the current investigator brochure.

As with every procedure risks and discomforts may occur with relations to the Breath ID test. The potential risks/complications and discomforts which may be associated with all study procedures are listed below.

- Allergic and/or other reactions to BreathID reagent
- Aspiration (inhaling) of food or fluids into the lungs
- Discomfort

When an adverse event occurs, the following information and assessments should be documented in the subject's file and reported in the adverse event section of the CRF:

- The signs, symptoms, or diagnosis of the event if available
- The date and time of onset of the event using the 24 hour clock where midnight is 00:00 and noon is 12:00
- The adverse event severity using the criteria outlined below
- The relationship of the event to the study medical device or drug as outlined below
- Describe any action taken regarding study medical device or drug disposition
- List any required therapy, medication, treatment, or diagnostic procedure.

17.3. Relationship to Study Medical Product

An investigator, who is a qualified physician, should assess the relationship to the study medical device and the substrate, based on all information available at the time of assessment.

The following definitions should be used:

- **Not Related:**

1. The event is clearly related to other factors such as the patient's clinical state, therapeutic interventions or concomitant drugs.
2. There is a highly likely alternative explanation.

- **Related:**

1. The event AE is reasonably associated with the use of the investigational product (device or substrate).

17.4. Adverse Event Severity

- **Mild Adverse Events**

A mild adverse event is one that the symptoms are barely noticeable to the patient. It does not influence performance, require drug treatment or prevent the patient from carrying on with normal life activities.

- **Moderate Adverse Events**

A moderate adverse event is one that the symptoms make the patient uncomfortable and causes some impairment to normal life activities. Treatment for symptom(s) may be required.

- **Severe Adverse Events**

A severe adverse event is one that the symptoms cause severe discomforts to the patient and severely limits the patient's normal daily activities. Treatment for symptom(s) is given.

Note: Serious and severe are not synonymous. A serious adverse event must fulfill the requirements listed in the definition above.

17.5. Adverse Event Reporting

The investigator should report all adverse events in the case report form. The investigator is responsible for the appropriate medical management of all adverse events.

The investigator must report any serious adverse event to the sponsor immediately, within 24 hours of awareness, via email. Full details of the event, severity, outcome (if available) and an assessment of the relationship to the study investigational product must be provided in the report.

All SAEs must be followed up until resolution or stabilization by submission of updated reports. Follow-up SAE reports will be reported according to the same timelines as initial reports, as soon as new significant information becomes available.

All SAEs that fulfill UADE or SUSAR definitions will be reported to the relevant authorities by expedited means.

Notification of the IECs/IRBs and regulatory authorities

Notification to the IECs/IRBs about all relevant events (e.g. SAEs, UADEs, SUSARs) will be performed by the sponsor and/or by the investigator according to all applicable regulations.

The processing and reporting of all relevant events to the regulatory authorities will be done by the sponsor according to all applicable regulations.

If unexpected safety issues are identified, specific amendments will be implemented.

18. ACCESS TO SOURCE DATA AND DOCUMENTS

18.1. Availability of Source Data and Documents

The Investigator will permit study-related monitoring, audits, IRB/EC review, and regulatory inspections (where appropriate) by providing direct access to source data and other documents (i.e. subjects' case sheets, blood test reports, histology reports etc.).

18.2. Subject Confidentiality

The subject's name and personal data will remain confidential and will not be published in any way. All data will be coded and stored in locked offices or on password protected media.

19. MONITORING AND QUALITY ASSURANCE

Monitoring of this study will be to ensure compliance with GCP, local regulations and scientific integrity and will be managed and oversight retained by Exalenz Bioscience (the Sponsor) or its assigned representative.

19.1. Data Collection and Monitoring

A set of electronic Case Report Forms (eCRF) will be completed for each subject. All case report forms will be filled out completely and will be reviewed for accuracy during monitoring.

The Investigator or designee will be responsible for recording study data into the eCRF provided by the Sponsor. It is the Investigator's responsibility to ensure the accuracy of the data entered into the eCRFs. The Sponsor or designee will be responsible for data processing, in accordance with data management procedures. Database lock will occur once monitoring and quality assurance procedures have been completed.

The eCRF will be managed based on CFR 21 Part 11 and EU's Annex 11.

20. PUBLICATION POLICY AND FINANCE

It is intended that the results of the study will be reported and disseminated at international conferences and in peer-reviewed scientific journals. The policy regarding publications appears in the non-disclosure agreement signed by each study investigator prior to signing of the study contract.

21. FINANCIAL ASPECTS

The device and compound will be provided by the Sponsor. Funding for regulatory approvals and administration will also be provided by the Sponsor. Funding will also be provided for one study support staff at local sites.

22. STUDY TERMINATION

The study may be terminated after appropriate consultation between the study sponsor and the principal investigator and co investigators. Conditions warranting termination include, but are not limited to:

- Failure of the investigator to enroll subjects at an acceptable rate
- Insufficient adherence to protocol requirements
- Failure to adhere to GCP
- Decision by the study sponsor to suspend or discontinue development of the device or kit

REFERENCES

1. Canete A, Abunaji Y, Alvarez-Calatayud G, DeVicente M, Gonzalez-Holguera JA, Leralta M, Pajares JM, et al. Breath test using a single 50-mg dose of 13C-urea to detect Helicobacter pylori infection in children. J Pediatr Gastroenterol Nutr 2003;36:105-111.
2. Levine A, Shevah O, Miloh T, Wine E, Niv Y, Bujanover Y, Avni Y, et al. Validation of a novel real time 13C urea breath test for rapid evaluation of Helicobacter pylori in children and adolescents. J Pediatr 2004;145:112-114.
3. Guarner J, Kalach N, Elitsur Y, Koletzko S. Helicobacter pylori diagnostic tests in children: review of the literature from 1999 to 2009. Eur J Pediatr 2010;169:15-25.
4. Chey WD, Wong BC. American College of Gastroenterology guideline on the management of Helicobacter pylori infection. Am J Gastroenterol 2007;102:1808-1825.
5. ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice. J Postgrad Med 2001;47:199-203.
6. Brenner H, Bode G, Adler G, Rothenbacher D. Does maternal smoking hinder mother-child transmission of Helicobacter pylori infection? Epidemiology 2000;11:71-75.
7. Drumm B, Rhoads JM, Stringer DA, Sherman PM, Ellis LE, Durie PR. Peptic ulcer disease in children: etiology, clinical findings, and clinical course. Pediatrics 1988;82:410-414.
8. Drumm B, Perez-Perez GI, Blaser MJ, Sherman PM. Intrafamilial clustering of Helicobacter pylori infection. N Engl J Med 1990;322:359-363.
9. Kilbridge PM, Dahms BB, Czinn SJ. Campylobacter pylori--associated gastritis and peptic ulcer disease in children. Am J Dis Child 1988;142:1149-1152.
10. Hino B, Eliakim R, Levine A, Sprecher H, Berkowitz D, Hartman C, Eshach-Adiv O, et al. Comparison of invasive and non-invasive tests diagnosis and monitoring of Helicobacter pylori infection in children. J Pediatr Gastroenterol Nutr 2004;39:519-523.
11. Beg MB, LeLeiko NS. Evaluation of the BreathID™ System for the Diagnosis of Helicobacter pylori Infection in Children. Journal of Pediatric Gastroenterology 2004;39 (Supplement I).
12. Paimela HM, Oksala NK, Kaariainen IP, Carlson PJ, Kostiala AA, Sipponen PI. Faecal antigen tests in the confirmation of the effect of Helicobacter eradication therapy. Ann Med 2006;38:352-356.
13. Malfertheiner P, Megraud F, O'Morain CA, Atherton J, Axon AT, Bazzoli F, Gensini GF, et al. Management of Helicobacter pylori infection--the Maastricht IV/ Florence Consensus Report. Gut 2012;61:646-664.
14. Korkmaz H, Kesli R, Karabagli P, Terzi Y. Comparison of the diagnostic accuracy of five different stool antigen tests for the diagnosis of Helicobacter pylori infection. Helicobacter 2013;18:384-391.
15. Zhou X, Su J, Xu G, Zhang G. Accuracy of stool antigen test for the diagnosis of Helicobacter pylori infection in children: a meta-analysis. Clin Res Hepatol Gastroenterol 2014;38:629-638.

16. Choi J, Kim CH, Kim D, Chung SJ, Song JH, Kang JM, Yang JI, et al. Prospective evaluation of a new stool antigen test for the detection of Helicobacter pylori, in comparison with histology, rapid urease test, (13)C-urea breath test, and serology. J Gastroenterol Hepatol 2011;26:1053-1059.
17. Gisbert JP, Cabrera Md Mdel M, Pajares JM. [Stool antigen test for initial Helicobacter pylori diagnosis and for confirmation of eradication after therapy]. Med Clin (Barc) 2002;118:401-404.
18. Weingart V, Russmann H, Koletzko S, Weingart J, Hochter W, Sackmann M. Sensitivity of a novel stool antigen test for detection of Helicobacter pylori in adult outpatients before and after eradication therapy. J Clin Microbiol 2004;42:1319-1321.
19. Bravo LE, Realpe JL, Campo C, Mera R, Correa P. Effects of acid suppression and bismuth medications on the performance of diagnostic tests for Helicobacter pylori infection. Am J Gastroenterol 1999;94:2380-2383.
20. Manes G, Balzano A, Iaquinto G, Ricci C, Piccirillo MM, Giardullo N, Todisco A, et al. Accuracy of the stool antigen test in the diagnosis of Helicobacter pylori infection before treatment and in patients on omeprazole therapy. Aliment Pharmacol Ther 2001;15:73-79.
21. Atherton JC. Non-endoscopic tests in the diagnosis of Helicobacter pylori infection. Aliment Pharmacol Ther 1997;11 Suppl 1:11-20.
22. Howden CW, Hunt RH. Guidelines for the management of Helicobacter pylori infection. Ad Hoc Committee on Practice Parameters of the American College of Gastroenterology. Am J Gastroenterol 1998;93:2330-2338.
23. Gisbert JP, Pajares JM. Review article: 13C-urea breath test in the diagnosis of Helicobacter pylori infection -- a critical review. Aliment Pharmacol Ther 2004;20:1001-1017.
24. Graham DY, Klein PD. Accurate diagnosis of Helicobacter pylori. 13C-urea breath test. Gastroenterol Clin North Am 2000;29:885-893, x.
25. Perri F. Diagnosis of Helicobacter pylori infection: which is the best test? The urea breath test. Dig Liver Dis 2000;32 Suppl 3:S196-198.
26. Chey WD. Accurate diagnosis of Helicobacter pylori. 14C-urea breath test. Gastroenterol Clin North Am 2000;29:895-902.
27. Hellinger A, Rauen U, de Groot H, Erhard J. [Auxiliary liver transplantation for acute liver failure after intake of 3,4-methylenedioxymethamphetamine ("Ecstasy")]. Dtsch Med Wochenschr 1997;122:716-720.
28. Pereira SP, McCarthy M, Ellis AJ, Wendon J, Portmann B, Rela M, Heaton N, et al. Auxiliary partial orthotopic liver transplantation for acute liver failure. J Hepatol 1997;26:1010-1017.

APPENDIX A - PROTOCOL APPROVAL SIGNATURE PAGE

Protocol No: **PED- HP- 0616B**

Protocol Title: **Clinical Study to Confirm Safety and Accuracy in Detection of H. pylori with ^{13}C-Urea Breath Test using the BreathID® Hp and BreathID® Hp Lab Systems in the Pediatric Population**

Version: 2.0

Date of Protocol: **March 12, 2017**

Site Name: _____

Principal Investigator: _____
Print name

I have read this protocol and agree to conduct the study as outlined herein and as per GCP and local regulations.

Principal/ Chief Investigator_____ **Date**_____

APPENDIX B – STOOL ANTIGEN TEST KIT AND PROCEDURE

The Premier Platinum HpSA PLUS enzyme immunoassay (EIA) is an in vitro qualitative procedure for the detection of *Helicobacter pylori* antigens in human stool. Test results are intended to aid in the diagnosis of *H. pylori* infection and to monitor response during and post-therapy in patients. Accepted medical practice recommends that testing by any current method, to confirm eradication, be done at least four weeks following completion of therapy.

Premier Platinum HpSA PLUS is a microwell-based enzyme immunoassay that detects *H. pylori* antigens present in human stool. No calculations are required and the visual color change makes the interpretation of results objective and simple. In addition, the HpSA test permits assessment of established or novel anti-*H. pylori* treatment during and post-therapy to monitor for treatment effectiveness, relapse or eradication. Premier Platinum HpSA PLUS is a modification of Premier Platinum HpSA that provides increased signal strengths with positive test results and better discrimination between low positive and negative tests.

The Premier Platinum HpSA PLUS test utilizes a plurality of monoclonal anti-*H. pylori* capture antibodies adsorbed to microwells. (Plurality is defined as a mixture of monoclonal antibodies.) Diluted patient samples and a conjugate (peroxidase conjugated to a plurality of monoclonal antibodies) are added to the wells and incubated for one hour at room temperature. A wash is performed to remove unbound material. Substrate is added and incubated for ten minutes at room temperature. Color develops in the presence of bound enzyme. Stop Solution is added and the results are interpreted visually or spectrophotometrically.

Procedure

The doctor or hospital laboratory usually will provide written instructions on how to collect a stool sample. If instructions aren't provided, here are tips for collecting a stool sample from your child:

- Be sure to wear protective gloves and wash your hands and your child's hands afterward.
- Some young kids can't always let a parent know in advance when a bowel movement is coming. So a hat-shaped plastic lid is used to collect the stool specimen. This catching device can be quickly placed over a toilet bowl, or under your child's bottom, to collect the sample. Using a catching device can prevent contamination of the stool by water and dirt. Another way to collect a stool sample is to loosely place plastic wrap over the seat of the toilet. Then place the stool sample in a clean, sealable container before taking it to the lab.
- Plastic wrap can also be used to line the diaper of an infant or toddler who isn't yet using the toilet. The wrap should be placed so that urine runs into the diaper, not the wrap. Stools shouldn't be allowed to touch the inside of disposable diapers because the lining usually has antibacterial properties that can interfere with the test results.

- Your child shouldn't urinate into the container. If possible, have your child empty his or her bladder before a bowel movement.
- The stool should be collected into clean, dry plastic jars with screw-cap lids. Your child may be asked to provide a stool sample one or more times. For best results, the stool should be brought to the lab right away. If this isn't possible, the stool should be refrigerated and then taken to the lab as soon as possible.
- Alternatively, a doctor or nurse may collect a small stool sample by inserting a swab into your child's rectum.

Getting the Results
In general, the result of the H. pylori stool test is reported in 1-4 days.

Interpretation of Results;
The following interpretations apply to both initial diagnosis and monitoring of anti- *H. pylori* therapy.

Visual Reading

Negative = colorless to faint yellow

Positive = definite yellow color

To be called positive, a faint yellow color must be confirmed by a spectrophotometric reading. If a spectrophotometer is not available, the cut-off must be determined by an alternative method.

Spectrophotometric Single Wavelength (450 nm)

Negative: < 0.140

Positive: ≥ 0.140

Negative Control: < 0.140

Positive Control: ≥ 0.640

Spectrophotometric Dual Wavelength (450/630 nm)

Negative: < 0.100

Positive: ≥ 0.100

Negative Control: < 0.100

Positive Control: ≥ 0.600

If a Negative Control is < 0.000, reblank the plate reader to air and reread the plate.

Reporting of Results:

A positive result indicates the presence of *H. pylori* antigens. A negative result indicates the absence of *H. pylori* antigens, or that the level of antigens is below what can be detected by the assay. The magnitude of the OD above the cut-off is not indicative of the severity or extent of *H. pylori* infection, nor can it be correlated to an endpoint titer. Extremely strong positive reactions may yield a purple precipitate within a few minutes of stopping the reaction.

Risks

No risks are associated with collecting stool samples.

Helping Your Child

Collecting a stool sample is painless. Tell your child that collecting the stool won't hurt, but it has to be done carefully. A child who's old enough might be able to collect the sample alone to avoid embarrassment. Tell your child how to do this properly. If the sample is collected by swabbing, your child may feel slight pressure in his or her rectum during the procedure.