ENVIVO BIO

CLINICAL TRIAL PROTOCOL

Protocol Number: EB-03

NCT05749068

An open-label study to evaluate the use of the CapScan intestinal collection device to measure the regional metabolism of sulfasalazine in the digestive tracts of healthy volunteers.

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Date of Protocol Version: 1.0 Dated: 5 May 2022

Revision	Date	Description of change
Rev 1.0	5 May 2022	First Issue

APPROVAL AND SIGNATURE PAGE

SPONSOR'S APPROVAL:

_____ **5/5/2022**
Dr. Dari Shalon **Date**
Envivo Bio Inc.
Project Manager

_____ **5/5/2022**
Pete Shabe **Date**
Biostatistics / Data Management

Version 1.0 – Dated: 5 May 2022

INVESTIGATOR'S STATEMENT

I agree to conduct the study as outlined in the protocol entitled: An open-label study to evaluate the use of the CapScan intestinal collection device to measure the regional metabolism of sulfasalazine in the digestive tracts of healthy volunteers in accordance with all applicable guidelines and government regulations.

I have read and understand all sections of the protocol.

George Triadafilopoulos, M.D.
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_____ 5/5/2022
Principal Investigator's Signature DATE

Version 1.0 - Dated: 5 May 2022

PROTOCOL SYNOPSIS

Title	An open-label study to evaluate the use of the CapScan® intestinal collection device to measure the regional metabolism of sulfasalazine in the digestive tracts of healthy volunteers.
Sponsor	Envivo Bio Inc.
Protocol No.	EB-03
Clinical Phase	Feasibility
Device	CapScan® intestinal sampling device
Indication	Healthy volunteers
Study Period	Total per-subject participation is up to 4 weeks
Study Objective	The objective is to evaluate the effectiveness of the CapScan intestinal collection device in characterizing the regional distribution of sulfasalazine, the metabolic breakdown products of sulfasalazine, and the gut microbiota in the digestive tracts of healthy volunteers.
Study Design	Prospective, open-label, single-arm study designed to provide effectiveness information on the CapScan intestinal collection device in healthy volunteers.
Planned Sample Size	This study will be conducted in up to 10 healthy subjects.

Inclusion Criteria	Subjects must meet all of the following criteria to be included in the study: Males or females 18 years of age or older and 70 years of age or younger at the time of the first Screening Visit. ASA Classification 1 or 2. For women of childbearing potential, negative urine pregnancy test within 7 days of Screening Visit and agreement to remain abstinent (refrain from heterosexual intercourse) or use adequate contraception during the treatment period and for 14 days after the final dose of sulfasalazine. Subject is fluent in English and understands the study protocol and informed consent and is willing and able to comply with study requirements and sign the informed consent form.

Exclusion Criteria	Subjects with any of the following conditions or characteristics must be excluded from the study: • Known or suspected gastrointestinal obstructions, strictures or fistula • Known or suspected moderate to severe dysmotility, by the judgement of the principal investigator • Gastroparesis • A swallowing disorder • Known intolerance, reaction or hypersensitivity to sulfasalazine, its metabolites, sulfonamides, or salicylates • Urinary obstruction • Porphyria • G6PD deficiency • Known or suspected infection with SARS-CoV2, HIV, HBV or HCV • Pregnancy or planned pregnancy within 30 days from Screening Visit, or breast-feeding. • Use of concomitant medications, such as aspirin, antibiotics, laxatives and proton pump inhibitors that may interfere with the study in the judgement of the investigator. • Any form of active substance abuse or dependence (including drug or alcohol abuse), unstable medical or psychiatric disorder, or any chronic condition susceptible, in the opinion of the principal investigator, to interfere with the conduct of the study. • A clinical condition that, in the judgment of the principal investigator, could potentially pose a health risk to the subject while involved in the study.
Device Description	The CapScan intestinal collection device is a single-use passive device that collects fluids from the gastrointestinal ("GI") tract for analysis outside the body. The CapScan Device contains no medicinal product. The CapScan Device is strictly a collection device.
Study Treatment	At the ***Screening*** Visit, study eligibility is confirmed and the subject signs the informed consent document after all questions have been appropriately answered. The subject is given 4 CapScan Devices and one 1,000 mg dose of sulfasalazine. The subject is provided with a snack bar to consume before swallowing the 4

	CapScan Devices. The subject is also given a retrieval wand and stool collection containers to snare the expelled CapScan Devices from the stool. The subject is also given 2 Tasso+ blood collection devices for home collection of blood samples, saliva and stool collection tubes for analysis of saliva and stool microbiome and metabolites. The blood, saliva and stool samples will be compared to the intestinal samples collected by the CapScan Device. The subject is given Daily Diaries to document the time the CapScan Devices were swallowed, what the subject ate, and time of bowel movements. On Day 1, the subject swallows 2 CapScan Devices and collects stool and saliva for the baseline set of measurements. On Day 2, the subject takes a single dose of 1,000 mg of sulfasalazine and swallows 2 CapScan Devices and collects 2 blood samples, plus stool and saliva. The timing of the dose of sulfasalazine, sampling of blood, and the ingestion of the CapScan Devices on each day will be at the discretion of the PI. The subject continues to collect all CapScan Devices expelled in the stool and stool samples until all ingested CapScan Devices have been retrieved, or until Day 8. In the event of missing CapScan Devices that were not successfully retrieved by the end of Day 8, and at the discretion of the PI, an abdominal x-ray will be performed on the subject to confirm no CapScan Devices are still in the body. In the event that a subject was unable to retrieve the 2 CapScan Devices swallowed on Day 2, or if the devices did not produce usable samples, the PI may request that the full set of procedures from Day 2 onwards will be repeated starting any day between Day 12-19 to give sufficient time for any sulfasalazine to be cleared from the body, including a second dose of 1,000 mg of sulfasalazine and swallowing and retrieval of the 2 additional CapScan Devices. The End of Study is defined when the last ingested CapScan Device is collected from the stool, or after determination by the PI that no CapScan Devices are in the subject's body. If a subject complains of any Adverse Event (AE) within 5 days of the End of Study that the investigator deems related to the device, the event will be documented and the subject will be evaluated by the principal investigator within 72 hours of the investigator becoming aware of the AE.

Endpoint	The endpoint of this trial is the regional measurement of sulfasalazine in the intestinal lumen, the metabolic breakdown products of sulfasalazine and the gut microbiota in the digestive tracts of healthy volunteers.
Safety Assessments	Safety assessments will include the following: ▪ Subject-reported adverse events, as communicated to the study staff. A medical evaluation, if necessary, to evaluate an adverse event.
Statistical methods	All measured variables and derived parameters will be listed individually and, if appropriate, tabulated by descriptive statistics.

List of Abbreviations and Terms

Abbreviation/Term	Definitions
ADE	Adverse device effect
AE	Adverse event
ASA	American Society of Anesthesiologists
BMI	Body mass index
BSLN	Baseline
CE	Conformité Européenne
EC	Ethics committee
Cm	Centimeter
CRF	Case report form
CRO	Contract research organization
GCP	Good Clinical Practice
ICF	Informed consent form
ICH	International Conference for Harmonisation
ISO	International Standard Organization
Kg	Kilogram
M	Meter
MedDRA	Medical Dictionary for Regulatory Activities
Mm	Millimeter
μl	Microliter
N	Number of subjects
PI	Principal investigator
SADE	Serious adverse device effect
SAE	Serious adverse event
SAP	Statistical analysis plan
SEM	Standard error of the mean
WHO	World Health Organization
USP	United States Pharmacopoeia
RFID	Radiofrequency Identification Device

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References

1. INTRODUCTION

It has recently been recognized that microbes, viruses, and micro-eukaryotes in the human gastrointestinal ("GI") tract perform many vital physiological functions that benefit their host organism, contributing to digestion, metabolizing drugs, producing essential amino acids and vitamins, regulating the immune system, providing resistance to disease, and even modifying appetite, and behavior.[i] Indeed it is estimated that the majority of drugs given orally today are metabolized or modified in some manner by the gut microbiome.[ii] Yet we know very little about the hundreds or thousands of microbial, viral, and micro-eukaryote species in the human GI tract ("microbiome").[iii] The variety of microbes in a single individual at different regions of the GI tract is staggering. Due to the complexity of this microbial ecology in a single individual and the variability among individuals, it is necessary to routinely sample and analyze the microbial community living along with its associated metabolites in all regions of the GI tract and measure their interactions with orally-delivered drugs.

The CapScan intestinal collection device ("CapScan Device") is a non-invasive sampling device that collects gastrointestinal samples along the GI tract that are then analyzed outside the body. One specific utility for this type of sampling is that the CapScan Device may enable the characterization and the performance of various drugs by detecting the presence of the drug and its metabolic breakdown products in the digestive lumen. Samples collected by the CapScan Device will undergo DNA sequencing and metabolite analysis. The goal will be to determine the identity and function of sulfasalazine, the metabolic breakdown products of sulfasalazine, and the gut microbes in the different regions of the GI tract, for comparison to similar analyses conducted on concomitantly collected saliva, blood and stool samples.

The ability to combine information about the identities and diversity of microbial community members obtained from these measurements could provide an important understanding of the microbiome's role in human physiology. Furthermore, understanding how to better characterize metabolic breakdown of drugs within the digestive tract may potentially improve the development and optimization of oral or enterally administered treatments for various diseases.

1.1 CURRENT APPROACHES

STOOL SAMPLING

The least invasive way to assess the microbiome is analyzing stool samples. However, stool samples do not capture the microbial activity at proximal sites of the intestine or in microbes

living close to the gut mucosa.[iv] Identification of microbes from stool reflects mainly the accumulated remnants of microbes that were more proximally active in the GI tract. Furthermore, many of the metabolites and proteins that are active in the small and large intestine are no longer present in the stool in their active form. Therefore, it is necessary to sample the microbiome directly from the stomach, small intestine and colon in order to accurately capture the full biochemical and immunological activity of the microbiome and its host interactions, and in order to better characterize the metabolic activity of drugs.

ENDOSCOPIC BIOPSIES

Endoscopic mucosal biopsies display greater microbial diversity than stool samples.[v] However, mucosal biopsies are associated with the invasiveness and risk of endoscopy, as well as the biopsy procedure that alters the mucosal surface of the GI tract.

To the best of our knowledge, no device exists to safely, routinely and non-invasively collect fluid samples from the various regions of the GI tract to provide highly relevant samples for microbiome and pharmocological function analysis.

BLOOD SAMPLES

Blood samples show how much of the drug is in systemic circulation. However, for drugs such as sulfasalazine that work in the gut lumen, circulating levels of the drug in blood may not be informative.

2. INVESTIGATIONAL DEVICE: CAPSCAN COLLECTION DEVICE

2.1 DEVICE DESCRIPTION

The CapScan Device is a single-use passive device that collects fluids from the gastrointestinal ("GI") tract for analysis outside the body.

The CapScan Device contains no medicinal product. The CapScan Device is strictly a collection device.

2.2 TREATMENT PROCEDURES

The subject is given 4 CapScan Devices and one 1,000 mg dose of sulfasalazine. The subject is provided with snack bars to consume before swallowing the CapScan Devices. The subject is given a retrieval wand to snare the expelled CapScan Devices from the toilet bowl and stool collection containers if they prefer to retrieve the device from stool in the container as opposed to from the toilet. The subject is also given 2 Tasso+ home-use blood collection devices, saliva

and stool collection tubes for analysis of blood, saliva and stool microbiome and metabolites. The blood, saliva and stool samples will be compared to the intestinal samples collected by the CapScan Device. The subject is given Daily Diaries to document the time the CapScan Devices were swallowed, blood and saliva samples collected, what the subject ate, and time of bowel movements.

On Day 1, after consuming a snack bar, the subject swallows 2 CapScan Devices and collects stool and saliva for the baseline set of measurements.

On Day 2, after consuming a snack bar, the subject takes a single dose of 1,000 mg of sulfasalazine and swallows 2 CapScan Devices and collects blood, stool and saliva.

The timing of the dose of sulfasalazine, sampling of blood, stool and saliva and the ingestion of the CapScan Devices on each day will be at the discretion of the PI.

The subject continues to collect all CapScan Devices expelled in the stool until all ingested CapScan Devices have been retrieved, or until Day 8. In the event of missing CapScan Devices that weren't successfully retrieved by the end of Day 8, and at the discretion of the PI, an abdominal x-ray will be performed on the subject to confirm no CapScan Devices are still in the body.

In the event that a subject was unable to retrieve the two CapScan Devices swallowed on Day 2 or if the devices did not produce usable samples, the PI may request that the full set of procedures from Day 2 will be repeated on one day between Day 12-19 to give sufficient time for any sulfasalazine to be cleared from the body, including a second dose of 1,000 mg of sulfasalazine and swallowing and retrieval of 2 additional CapScan Devices. The End of Study is defined when the last ingested CapScan Device is collected from the stool, or after determination by the PI that no CapScan Devices are in the subject's body.

If a subject complains of any Adverse Event (AE) within 5 days of the End of Study that the investigator deems related to the device, the event will be documented and the subject will be evaluated by the principal investigator or sub investigator within 72 hours of either investigator becoming aware of the AE.

2.3 DEVICE INTENDED USE

The CapScan Device is intended to collect liquid samples from the small intestine and colon.

2.4 RISK BENEFIT ANALYSIS

2.4.1 Possible Risks

The potential risks associated with the use of CapScan Device include transient discomfort when swallowing a CapScan Device. Additional risks include aspiration and intestinal blockage.

The risks above are considered reasonable given the following safeguards:

Physically, the size and shape of the CapScan Device is similar to a standard size 00 medicinal capsule. As such, the swallowing of the CapScan Device does not pose any greater risk to the health, safety, or welfare of the patient than the swallowing of a standard medicinal capsule.

The passage of the CapScan Device through the GI tract does not present any greater risk to the health, safety, or welfare of the patient than a Medtronic Pillcam®, which is routinely swallowed for diagnostic purposes and eliminated via defecation. The Pillcam® is hard and inflexible and is 12 mm in diameter, *versus* the CapScan Device which is soft and flexible and only 6 mm in diameter. A radio-opaque marker incorporated into the CapScan Device enables definitive confirmation that the device has been eliminated from the body, if necessary.

Generally, healthy volunteers with any underlying intestinal stricture that would prevent the passage of items 6 mm in diameter through the GI tract would be symptomatic even before the study. Examples of symptoms of pre-existing intestinal blockage could include pain and bloating after eating corn or other vegetables, for example.

In the unexpected event that the CapScan Device is retained in the intestines, the CapScan Device can be removed via motility enhancers (i.e. laxatives) or via laparoscopic surgery in a worst case scenario. In the event of a retained CapScan Device, there is a potential benefit for the subject to discover and treat the underlying cause of any pre-existing intestinal strictures inadvertently discovered as part of this study.

All of the materials in the CapScan Device have been well characterized and understood to be biocompatible and used in a variety of cleared medical products and pharmaceuticals.

Sulfasalazine is an FDA-approved drug indicated for treatment of ulcerative colitis and rheumatoid arthitis. In clinical practice, sulfasalazine is most commonly associated with the following side effects: gastric distress, headache, nausea, oligospermia (temporary reduced sperm count), vomiting, and anorexia. Less frequent side effects include pruritis (itching), urticaria (hives), rash, fever, Heinz body anemia, hemolytic anemia, and cyanosis.

Research and clinical experience suggests that side effects are more frequent in individuals taking daily doses of 4,000 mg or higher and for prolonged periods of time (months to years). Due to the relatively low single dose of 1,000 mg sulfasalazine administered in this study, side effects are expected to be rare and less serious. Regardless, subjects will be instructed to report any experienced side effects to the study investigator.

Finally, the proposed clinical investigation does not involve any anticipated surgical procedures, nor do any of the other clinical procedures proposed for use during the study present a potential for serious risk to the health, safety, or welfare of a subject.

2.4.2 Possible Benefits

The possible benefits offered by the CapScan Device for future patients might be the development of better predictive models on the effectiveness of sulfasalazine and/or other drug therapies, disease diagnosis, patient stratification, and potentially the development of transformative treatments for various diseases.

2.4.3 Alternative Treatments Summary

Alternative approaches for collecting samples from the GI tract and looking for drug pharmacokinetics, include measuring the drug levels in the blood and/or stool samples, which are not representative of the actual luminal drug levels or and only offer limited insight into effects of the gut microbiome on drug metabolism.

2.4.4 Safety Measures Taken

Physically, the size and shape of the CapScan Device is similar to a standard size 00 medicinal capsule. As such, the swallowing of the CapScan Device does not present any greater risk to the health, safety, or welfare of the patient than the swallowing of a standard medicinal capsule.

The passage of the CapScan Device through the GI tract does not present any greater risk to the health, safety, or welfare of the patient than a Given Imaging Pillcam®, which is routinely swallowed and eliminated via defecation and is significantly harder, larger and more voluminous than the CapScan Device. The radio-opaque marker incorporated into the CapScan Device enables definitive confirmation that the device has been eliminated from the body if necessary.

All of the materials in the CapScan Device are well characterized and understood to be biocompatible and used in a variety of cleared medical products and pharmaceuticals.

The considerations mentioned above have been taken during the design and manufacturing of the CapScan Device to ensure its safe and reliable performance.

The selection of and dosage of sulfasalazine is intended to minimize observed side effects related to the drug used for metabolic analysis.

3. STUDY RATIONALE

Performing drug metabolism and microbiome analyses on stool samples does not fully represent the diversity of the microbial, viral and micro-eukaryotic species, metabolism and host interactions occurring in the intestines. Obtaining samples via endoscopic biopsy is invasive and not convenient. Understanding drug levels in the lumen of the gut will provide insight into drug

metabolism and pharmacokinetics as well as enable an understanding of systemic versus intestinal lumen drug concentrations.

The CapScan Device is designed to be a safe and convenient method for obtaining gastrointestinal samples from key regions of the GI tract for microbiome analysis. This study is focused on examining the safety and efficiency of sample collection of the CapScan Device in humans, as well as its suitability for adequate sample collection process aimed at subsequent DNA, metabolite and drug pharmacokinetics and metabolism analyses. Sulfasalazine was selected as the drug for metabolic characterization based on its safety profile and low likelihood of causing harm to healthy subjects when ingested for analytical purposes. Furthermore, sulfasalazine is degraded by gut microbes into known metabolites, although to the best of our knowledge, this process has not been observed directly in the human gut before.

The trial sample size is considered the minimal size required to collect sample collection efficiency data.

4. STUDY OBJECTIVES

The objective is to evaluate the effectiveness of the CapScan Device for characterizing the regional distribution of sulfasalazine, the metabolic breakdown products of sulfasalazine and the gut microbiota in the digestive tracts of healthy volunteers.

5. STUDY DESIGN

This will be an open-label feasibility study conducted in up to 10 subjects at a single center.

All subjects will undergo similar pre-study screening and evaluation and study procedures.

SCREENING VISIT:

During the Screening Visit, study eligibility is confirmed, the informed consent process is properly conducted and documented and instructions are given for use of the CapScan Device, saliva and stool collection, ingestion of sulfasalazine, and completion of the Daily Diaries.

The CapScan Devices are provided in individually-sealed containers.

DAY 1:

2 CapScan Devices are swallowed on Day 1. The subject will eat a study-provided snack bar and swallow the CapScan Devices at times of the day that are at the discretion of the PI. The subject also collects saliva and stool samples using the collection kits provided to them.

The subject uses a retrieval wand to snare any CapScan Devices that are expelled together with the stool.

The retrieved CapScan Devices, saliva and stool samples are stored in closed containers inside a study-supplied refrigerator until collected by the study staff.

The subject will complete a daily diary to document the time the CapScan Devices were swallowed, what they ate, when they sampled saliva, when they had bowel movements and the time the CapScan Devices were recovered, if any were recovered.

DAY 2:

Two CapScan Devices are swallowed on Day 2. The subject will also eat a study-provided snack bar and take the physician-directed dose of 1,000 mg of sulfasalazine. The subject also collects up to two samples of blood using the Tasso+ device, saliva and stool samples using the collection kits provided to them. The timing of the above procedures will be at the discretion of the PI.

The subject will retrieve the CapScan Devices from the stool using the retrieval wand provided by the study sponsor.

The subject will complete a daily diary to document the time the CapScan Devices were swallowed, what they ate, when they swallowed the sulfasalazine, sampled blood and saliva, had bowel movements and the time the CapScan Devices were recovered, if any were recovered.

DAYS 3-8:

The subject will continue to retrieve the CapScan Devices, collect stool and complete the daily diaries until the last device is retrieved. If not all devices have been retrieved by Day 8, the PI may elect to perform an abdominal x-ray to confirm that no CapScan Devices are still in the body. The End of Study is defined when the last ingested CapScan Device is collected from the stool, or after determination by the PI that no CapScan Devices are in the subject's body.

DAYS 12-19, if needed:

In the event that a subject was unable to retrieve the two CapScan Devices swallowed on Day 2 or if the devices did not produce usable samples, the PI may request that the full set of procedures from Day 2-8 will be repeated starting on any day between Day 12-19 to give sufficient time for any sulfasalazine to be cleared from the body, including a second dose of 1,000 mg of sulfasalazine and swallowing and retrieval of 2 additional CapScan Devices. The end of the study is defined when the last swallowed CapScan Device is retrieved from the stool or after determination by the PI that no CapScan Devices are in the subject's body. At this point, a study representative will go to the subject's house to collect all unused CapScan Devices,

retrieved devices, blood, saliva and stool samples, refrigerator and the Daily Diaries from the subject.

6. STUDY POPULATION

Study subjects will be healthy volunteers (ASA classes 1 and 2).

6.1 INCLUSION CRITERIA

Subjects must meet all of the following criteria to be included in the study:

- Males or females 18 years of age or older and 70 years of age or younger at the time of the first Screening Visit.
- ASA Classification 1 or 2.
- For women of childbearing potential, negative urine pregnancy test within 7 days of Screening Visit and agreement to remain abstinent (refrain from heterosexual intercourse) or use adequate contraception during the treatment period and for 14 days after the final dose of sulfasalazine.
- Subject is fluent in English and understands the study protocol and informed consent and is willing and able to comply with study requirements and sign the informed consent form.

6.2 EXCLUSION CRITERIA

Subjects with any of the following conditions or characteristics must be excluded from the study:

- Known or suspected gastrointestinal obstructions, strictures or fistula
- Known or suspected moderate to severe dysmotility, by the judgement of the principal investigator
- Gastroparesis
- A swallowing disorder
- Known intolerance, reaction or hypersensitivity to sulfasalazine, its metabolites, sulfonamides, or salicylates
- Urinary obstruction
- Porphyria
- G6PD deficiency
- Known or suspected infection with SARS-CoV2, HIV, HBV or HCV
- Pregnancy or planned pregnancy within 30 days from Screening Visit, or breast-feeding.

- Use of concomitant medications, such as aspirin, antibiotics, laxatives and proton pump inhibitors that may interfere with the study in the judgement of the investigator.
- Any form of active substance abuse or dependence (including drug or alcohol abuse), unstable medical or psychiatric disorder, or any chronic condition susceptible, in the opinion of the principal investigator, to interfere with the conduct of the study.
- A clinical condition that, in the judgment of the principal investigator, could potentially pose a health risk to the subject while involved in the study.

6.3 SUBJECT IDENTIFICATION

A unique subject identifying number will be assigned when an individual subject is qualified for enrollment. Before formal enrollment into the study, subjects will be uniquely identified by their initials and a subject identification number only.

6.4 REMOVAL, REPLACEMENT, OR EARLY WITHDRAWAL OF SUBJECTS FROM THERAPY OR ASSESSMENT

Subjects are free to discontinue their participation in the study at any time and without prejudice to further treatment, up until the time of publication of the study results, at which point withdrawal from the study is no longer possible. The Principal Investigator (PI) must withdraw any subject from the study, if that subject requests to be withdrawn. If a subject is withdrawn from the study or fails to return either at his or her request or at the PI's sole discretion, every effort should be made to determine the reason. This information will be recorded in the subject's CRF.

When a subject is removed from the study for a Serious Adverse Event, a final medical evaluation must be performed. Patients removed from the study because of an adverse event will be followed-up until the adverse event has resolved or stabilized.

If a subject has discontinued study participation during the CapScan Device Use Phase, the subject will return all collected and unused CapScan Devices, collected saliva, blood and stool samples, the completed Daily Diaries and the refrigerator.

Subjects withdrawn from the study will be replaced, regardless of the reason for withdrawal.

The subject's participation in this study may be discontinued due to any of the following reasons:

- Failure to follow the instructions of the PI and study staff
- The PI decides that continuing participation could be harmful to the subject
- Pregnancy
- The study is cancelled

- Other administrative reasons
- Unanticipated circumstances

6.5 HANDLING OF WITHDRAWALS

If a subject is withdrawn from the study or fails to return either at his or her request or at the PI's sole discretion, every effort should be made to determine the reason. This information will be recorded on the subject's case report form (CRF).

All subjects who withdraw from the study prematurely, regardless of cause, should undergo all early termination assessments; if relevant. It is vital to obtain follow-up data for any subject withdrawn due to an AE following device use.

6.6 SPONSOR'S TERMINATION OF STUDY

Envivo Bio Inc. reserves the unilateral right to discontinue the study at any time for any reason. Envivo Bio Inc. may also discontinue the study for inadequate site performance or compliance. Such a termination must be implemented by the PI, if instructed to do so by Envivo Bio Inc., in a time frame that is compatible with the subjects' well-being.

7. STUDY PROCEDURES

7.1 STUDY PROCEDURES CHART

A schedule of study procedures for the investigator and study subject are shown in Table 1 and Table 2 respectively. No protocol related procedures will be performed before subjects review and sign a written informed consent form.

Table 1: SCHEDULE OF **INVESTIGATOR** STUDY PROCEDURES

PROCEDURES	**SCREENING** 1 Day	**CAPSCAN DEVICE USE** 2 days	**CAPSCAN DEVICE RETRIEVAL** Up to 8 days
Informed Consent	X		
Inclusion/Exclusion	X		
Demographics	X		
Medical History	X		
Concomitant Medications	X		
General Health Assessment (ASA classification)	X		
Urine Pregnancy	X		
AE/SAE		X	X

Table 2: SCHEDULE OF **SUBJECT** STUDY PROCEDURES			
PROCEDURES	**SCREENING** Day 1	**CAPSCAN DEVICE USE** Day 2	**CAPSCAN DEVICE RETRIEVAL** Days 3-8
Informed Consent	X		
Swallows 2 CapScan Devices	X	X	
Collect saliva samples when swallowing CapScan Devices	X	X	
Collect blood using Tasso+ home-use tests		X	
Retrieve CapScan Devices from stool using retrieval wand	X	X	X
Ingests 1000mg of sulfasalazine		X	
Complete Daily Diaries to document the time the CapScan devices were swallowed, what was eaten, and time of bowel movements	X	X	X

7.2 SCREENING VISIT

The purpose and procedures of the study will be fully explained to potential subjects. Those individuals wishing to enroll in the study will sign a written informed consent form prior to initiating any evaluations or procedures. The Screening Visit includes:

- Review and sign Informed Consent by subject and PI or PI designee
- Assess suitability of subject to enroll in the study according to the Inclusion and Exclusion Criteria
- Obtain subjects medical history (including clinically significant abnormalities of all body systems), demographic data and concomitant medications
- Conduct health assessment of subject to include ASA classification
- Provide instruction for and dispense Daily Diaries to complete at home.

- Provide instruction for use and dispense, or have the Sponsor dispense, 4 CapScan Devices.
- Provide instructions for use and dispense, or have the Sponsor dispense, blood collection devices, retrieval wands, stool and saliva collection containers for collecting CapScan Devices expelled in stool.
- Provide instruction for and dispense, or have the Sponsor dispense, saliva and blood collection kits.
- Provide instructions for use and dispense, or have the Sponsor dispense, 1,000 mg of sulfasalazine.
- Assign subject a unique study number.

7.3 CAPSCAN DEVICE USE PHASE

- The CapScan Devices are provided in a sealed containers.
- The subject uses a device retrieval wand to look for and collect the expelled devices in the stool after all bowel movements for up to 8 days after swallowing a CapScan Device.
- The end of the study is defined when the last swallowed CapScan Device is retrieved from the stool. The study representative will collect all of the retrieved samples and the Daily Diaries from home of the subject.

7.3 PROTOCOL AMENDMENTS, REVISION AND/OR DEVIATIONS

The protocol must be read thoroughly and its instructions and guidelines must be followed exactly. Any changes in the protocol will require a formal amendment document. Such amendments will be agreed upon and approved in writing by the PI and Envivo Bio Inc. All modifications and revisions will be noted and summarized on page 1 of this protocol document. The Institutional Review Board (IRB) will be notified of all significant amendments to the protocol. Significant amendments to the protocol that may increase risk to the subject, and/or that may adversely affect the rights of the subject or the validity of the investigation will not be implemented until IRB committee written approval has been received.

With the exception of emergency situations, no significant changes or deviations in the conduct of this protocol will be permitted without the prior documented approval of Envivo Bio Inc. The IRB that granted the original approval for the study must be notified of all material changes in the protocol, and must provide a written approval of any significant change or significant deviation that may increase risk to the subject, and/or that may adversely affect the rights of the subject or the validity of the investigation.

In the event of an emergency, the PI will institute any medical procedures deemed appropriate. However, all such procedures must be promptly reported to Envivo Bio Inc. and the IRB.

7.4 MANUFACTURING AND SUPPLY OF INVESTIGATIONAL DEVICE

Envivo Bio Inc. will supply the CapScan Devices to the investigational site or subjects in appropriately labeled packaging.

The CapScan Device will be manufactured and packaged by Envivo Bio Inc. and/or its designated sub-contractors in compliance with the relevant principles and guidelines applicable to investigational medical devices.

7.5 STORAGE AND ACCOUNTABILITY OF INVESTIGATIONAL DEVICE

The CapScan Devices will be kept in a secure, limited-access, room temperature storage area at the investigational site or dispensed directly to the subjects by the Sponsor. Only authorized personnel will have access to the study devices. Complete traceability records will be kept of all devices during the study. Specifically, a study accountability log will be completed to maintain accurate records of all CapScan Device inventory.

7.6 RETURN OF INVESTIGATIONAL DEVICE

Government regulations require that all investigational product materials not used in clinical trials be returned to the Sponsor at the completion of the study. The monitor will be responsible for the accountability of the returned of used and unused investigational devices.

7.7 CONTRA-INDICATION

No contraindications related to the CapScan Device are currently known.

8. STUDY ASSESSMENT METHODS

8.1 SAFETY ASSESSMENTS

8.1.1 Adverse Events

Adverse Events (AE's) reported by subjects or observed by the PI or PI designee will be documented in the source document, assessed by the PI and will be individually listed on an adverse event page in the CRF. Recording of AE's will begin immediately after the signing of the informed consent document and continue until the subject dies, withdraws consent, or 48 hours after the End of Study (EOS), regardless of the relationship of the AE to the CapScan Device. If a subject complains of any AE within 5 days of the EOS that the PI deems definitely, probably, or possibly related to the device, the event will be documented and followed until all parameters

(including laboratory) have returned to normal, stabilized, or are otherwise explained. The subject will be evaluated by the PI within 72 hours of the PI becoming aware of the AE.

Information to be collected includes type of event, date of onset, date of resolution, investigator-specified assessment of severity and relationship to device, seriousness, as well as any action taken.

All conditions present before the Screening Visit will be documented as medical history.

Specific procedures for recording and reporting serious adverse events are described in section 10.5.

8.1.2 General Health Assessment

General health of the subject will be assessed at the Screening Visit utilizing the American Society of Anesthesiologists (ASA) Physical Status Classification System. Only subjects with an ASA classification of 1 and 2 will be eligible to participate in the trial.

- ASA Physical Status **1** - A normal healthy patient
- ASA Physical Status **2** - A patient with mild systemic disease
- ASA Physical Status **3** - A patient with severe systemic disease
- ASA Physical Status **4** - A patient with severe systemic disease that is a constant threat to life
- ASA Physical Status **5** - A moribund patient who is not expected to survive without the operation
- ASA Physical Status **6** - A declared brain-dead patient whose organs are being removed for donor purposes

9. STUDY ENDPOINTS

The study's endpoint is the regional measurement of sulfasalazine, the metabolic breakdown products of sulfasalazine in the digestive tract and peripheral circulation, and the gut microbiota in the digestive tracts of healthy volunteers.

Safety assessments will include the monitoring of subject-reported adverse events as communicated to the study staff.

10. SAFETY

10.1 Definition of Adverse Event

An adverse event (AE) is any untoward, undesirable, unplanned clinical event in the form of signs, symptoms, or clinical significant deviation from baseline in laboratory or physiological observations occurring in a human being participating in a clinical study with a Sponsor study device regardless of a causal relationship to device or device delivery and removal procedures. This includes the following:

- Any clinically significant worsening of a pre-existing condition;
- Any reoccurrence of a pre-existing condition;
- An AE that has been associated with the premature removal of the device.

An adverse device effect (ADE) is any untoward and unintended response to the investigational device or device delivery and removal procedures.

10.2 DEFINITION OF SERIOUS ADVERSE EVENT

A **serious** adverse event (SAE) is any adverse event that suggest a significant hazard or side effect, regardless of the PI's or Sponsor's opinion on the relationship to the investigational product and that results in, but may not be limited to, any of the following outcomes:

- death (regardless of the cause)
- a life-threatening event
- hospitalization or prolongation of existing hospitalization (any inpatient hospital admission that includes a minimum of an overnight stay in a health care facility)
- a persistent or significant disability/incapacity
- a congenital anomaly or birth defect
- significant medical events which requires medical intervention to prevent any of the above outcomes

Significant medical events are those which may not be immediately life-threatening, but may jeopardize the subject and may require intervention to prevent one of the other serious outcomes listed above.

Hospitalization for elective treatment of a pre-study condition that did not worsen while on study and hospitalizations for treatment of non-adverse events (e.g. cosmetic surgery) are not considered serious adverse events.

An **unexpected** adverse medical device event is any adverse event, the specificity or severity of which is not consistent with information in the current Investigator's Brochure (IB) for an unapproved medical device.

A **serious adverse device effect** (SADE) is any ADE that has resulted in any consequences characteristics of a serious adverse event or that might have led to any of these consequences if suitable action had not been taken or intervention had not been made or if circumstances had been less opportune.

10.3 DEFINITION OF RELATIONSHIP TO INVESTIGATIONAL DEVICE AND DEVICE DELIVERY AND REMOVAL PROCEDURES

The PI will document his/her clinical judgment of the causal relationship of the investigational product to the AE. The PI should consider and evaluate alternative causes, such as underlying conditions or other risk factors, as well as the temporal relationship of the event and the study intervention in their assessment of causality. The AE relationship will be defined using the criteria outlined in Table 4.

Table 4: Definition of Adverse Event Relationship Criteria

Relationship Criteria	Definition
PROBABLY	The adverse event is most likely to be explained by the investigational device or device delivery procedure, rather than the subject's clinical state or other agents/therapies; as the event follows a reasonable temporal sequence from the medical device delivery timepoint and it abates upon removal of the medical device.
POSSIBLY	The adverse event may be explained by the investigational device or device delivery procedure, or the subject's clinical state or other agents/therapies.
UNRELATED	The adverse event can be explained by the subject's clinical state or other agents/therapies.

10.4 DEFINITION OF SEVERITY OF ADVERSE EVENTS

Adverse events must be graded for severity. A severity category of mild, moderate, severe or life threatening as defined below, must be entered on the AE CRF page.

1. MILD – Awareness of signs or symptoms, but they are easily tolerated and no disruption of normal daily activity.
2. MODERATE – Enough discomfort to cause interference with normal daily activity. No treatment or medical intervention is indicated.
3. SEVERE – Inability to work or perform normal daily activity; medical intervention is indicated.
4. LIFE THREATENING/DISABLING – An immediate threat to life or leading to a permanent mental or physical conditions that prevents work or performing normal daily activities. Treatment or medical intervention is required in order to maintain survival.

10.5 REPORTING OF ADVERSE EVENTS

Any Serious adverse event (SAE) and device respectively procedure-related (Relatedness: definite, probably and possibly) Adverse Effect (SADE), must be fully recorded utililzing the sponsor provided SAE form and reported in writing by the Principal Investigator to Envivo Bio Inc. within 24 hours of becoming aware of the event.

The PI will complete the Serious Adverse Event Form and email a scanned copy to Envivo Bio Inc. sponsor and the medical monitor contacts as follows:

Additional information (follow-up) about any SAE or SADE unavailable at the initial reporting should be forwarded by the site to the Envivo Bio Inc. as soon as they become available (such as hospital discharge).

Envivo Bio Inc. will review and assess with the PI any SAE's and any serious device/procedure-related adverse effects. Envivo Bio Inc. ensures that reporting requirements according to the local regulations are fulfilled.

All SAE's which are considered as device- or procedure-related will be reported to IRB by the PI.

Subjects who have had an SAE during the Treatment Phase must be followed clinically until all parameters (including laboratory) have returned to normal, have stabilized, or are otherwise explained. The study period for the purpose of SAE reporting is defined as the period from time of informed consent until 14 days after the End of Study.

If the investigator detects a SAE in a trial subject after the end of the period of observation, and considers the event possibly related to the prior trial, he should contact the sponsor to determine how the AE should be documented and reported.

10.6 DOCUMENTATION OF AEs

All AEs reported by the subject or detected by the investigator will be documented on the appropriate pages of the case report form (CRF). AEs must also be fully documented in the subject's source documents.

If the adverse event is serious, the investigator must complete, in addition to the "Adverse Event" page, a "Serious Adverse Event" Report Form at the time the serious adverse event is detected.

Every attempt should be made to describe the adverse event in terms of a diagnosis. If a clear diagnosis has been made, individual signs and symptoms will not be recorded unless they represent atypical or extreme manifestations of the diagnosis, in which case they should be reported as separate events. If a clear diagnosis cannot be established, each sign and symptom must be recorded individually.

10.7 PREGNANCY

Female subjects should immediately inform the Investigator of any pregnancies during the study. The site should report all pregancies within 48 hours to the Envivo Bio Inc. Project Manager using the SAE form.

11. STATISTICAL ANALYSIS PLAN

Due to the small sample size and the study objectives, the statistics will be mainly descriptive by nature. For categorical variables, summary tables will be provided giving sample size, absolute

and relative frequency. For continuous variables summary tables will be provided giving sample size, arithmetic mean, standard deviation, standard error of the mean (SEM), median, minimum and maximum, percentiles and 95% CI (Confidence Interval) for means of variables.

Tables of descriptive statistics will be presented for the following variables:

- Adverse events and device-related adverse events in the clinical documentation.
- Effectiveness of the CapScan Device in collecting measurable quantities of metabolic breakdown products of sulfasalazine and for the identification of microbial enzyme genes.

All safety data will be summarized across the cohort with descriptive statistics. The incidence of adverse events during the treatment period will be summarized.

All adverse events will be classified by System Organ Class and preferred term according to the Medical Dictionary for Regulatory Activities (MedDRA). The latest version available at the time of coding will be used throughout the study.

All adverse events will be listed together with information on onset, duration, frequency, severity, seriousness, relationship to the investigational product, outcome and action taken. Frequency tables for severity and relationship to the Envivo Bio Device device will be provided by System Organ Class and preferred term.

The main analysis will not include events which started before the first use of CapScan Device number 1; only treatment emergent adverse events will be included in the summary tables.

VAS scores and changes from baseline will be presented by appropriate summary tables. Changes from baseline will be analyzed using Paired T-test or Signed rank test for two means (paired observations, or as appropriate).

Any deviation from the statistical analysis plan will be recorded together with an explanation of the deviation.

The data will be analyzed using standard statistical software (e.g., SAS [Cary, NC] or R [Vienna, Austria]).

12. SAMPLE SIZE CONSIDERATION

10 healthy subjects will be included in this study. The planned sample size was considered adequate by the Sponsor for this feasibility study. The study is not expected to show statistical significance or statistical power, but only to demonstrate safety and a trend for effectiveness and suitability of the sample collection process.

13. ETHICS

12.1 INSTITUTIONAL REVIEW BOARD

Prior to initiation of the study, the PI will submit the study protocol and significant amendments, sample Informed Consent Form (ICF), and any other documents that may be requested to the Instituional Review Board (IRB) for review and approval. The PI will request that the IRB provide written approval of the study and will keep on file records of approval of all documents pertaining to this study. The Investigator will not begin the study until the protocol and ICF have been approved by the IRB. The PI must agree to make any required progress reports to the IRB, as well as reports of device-related SAEs, life-threatening problems, or death. The PI will provide a copy of the written IRB approval of the protocol and ICF to the Sponsor prior to the start of the study.

12.2 ETHICAL CONDUCT OF THE STUDY

This trial will be conducted in compliance with this protocol, Sponsor instruction, the guidelines for good clinical practice (GCP), ISO 14155 parts 1 and 2 (2003), Medical Device Directive 93/42/EEC, Declaration of Helsinki; and applicable federal and local regulatory requirements.

All clinical work conducted under this protocol is subject to GCP rules. This includes an inspection by Envivo Bio Inc. or its designee, health authority, IRB representatives at any time. The PI must agree to the inspection of study-related records by health authority representatives and/or Envivo Bio Inc. or its designee.

12.3 SUBJECT INFORMATION AND CONSENT

At the Screening Visit the subject will be informed in detail about the investigational CapScan Device, and the nature of the clinical investigation with known or foreseeable benefits, risks and discomforts that subject may experience. The basic elements of informed consent as specified by ICH-GCP will be followed.

Written informed consent will be obtained from each subject to be involved in the clinical trial by using the Informed Consent Form (ICF) prior to the conduct of any study-related activity. Each subject will be given a copy of the written ICF. The subject must understand that throughout the study his or her participation remains voluntary and protected by the Declaration of Helsinki. The investigator is responsible for obtaining written (or witnessed) informed consent from potential subjects prior to study entry.

Subjects will be given time to read the informed consent and ask any questions before being asked to sign the form. The subjects will also be instructed that they are free to withdraw their consent and discontinue their participation in the study at any time without prejudice. If a particpant withdraws during the Treatment Phase, the investigator will schedude an Early Termination visit.

Prior to the start of the study, the PI or PI designee will provide Envivo Bio Inc. with a blank copy of the ICF version approved by the IRB. Each subject's study file will include the signed ICF for study participation.

When the study Treatment Phase is completed and the CRF has been monitored, the ICF will be kept in the investigator's central study file for the required period of time.

12.4 SUBJECT INSURANCE

The sponsor has subscribed to an insurance policy covering, in its terms and provisions, its legal liability for injuries caused to participating persons and arising out of this research performed strictly in accordance with the scientific protocol as well as with applicable law and professional standards. The insurance was taken out with a maximum limit of $1,000,000 per participating person.

Any impairment of health which might occur in consequence of trial participation must be notified to the insurance company. The study subject is responsible for notification. The insured person will agree to all appropriate measures intended to clarify the cause and the extent of damage as well as the reduction of damage.

During the conduct of the trial, the study subject must **not** undergo other clinical investigational treatment except for cases of emergency. The study subject is bound to inform the investigator immediately about any AE's and additionally drugs taken. The terms and conditions of the insurance should be delivered to the study subject.

12.5 INFORMING THE GENERAL PRACTITIONER

The PI should inform the study subject's regular physician of his/her participation in this study, by sending a letter to the physician, if the study subject agrees.

12.6 PERSONAL DATA PROTECTION

Envivo Bio Inc. complies with the principle of subject's right to protection against invasion of privacy. Throughout this trial, all data will be identified only by an identification number and subject initials. The data will be blinded in all data analyses. The study subject must be informed and consent is required that authorised personnel of Envivo Bio Inc. (Study Monitor, Auditor, Investors and Consultants etc.) and relevant Health regulatory agency will have direct access to personal medical data to assure a high quality standard of the study.

14. QUALITY CONTROL AND QUALITY ASSURANCE

Envivo Bio Inc. maintains quality assurance to ensure that clinical trials are conducted and data are generated, documented and reported in compliance with the protocol, GCP and applicable regulatory requirments.

This clinical trial may be audited by the Sponsor to determine whether the study is being conducted and monitored in compliance with the protocol as well as recognized GCP guidelines and local regulations. These audits will also increase the likelihood that the study data and all other study documentation can withstand a subsequent inspection by any regulatory authority.

Such audits, if necessary, will be pre-arranged with the site and conducted within a reasonable period.

The study may also be inspected by regulatory agencies. These inspections may take place at any time during or after the study and are based on the federal regulations, as well as ICH-GCP guidelines.

13.1 STUDY MONITORING

Monitoring of the study is the responsibility of the Sponsor. The study monitor will advise the PI regarding the practical conduct of the study and maintaining compliance with the protocol, GCP and all applicable regulatory requirements. Throughout the course of the study, the Sponsor and the study monitor will oversee the conduct and the progress of the study by frequent contacts with the investigator. This will include telephone calls and on-site visits. During the on-site visits, the CRFs will be reviewed for completeness with corresponding source documents. As part of the routine data monitoring, source documents will be made available for review by the study monitor. The study monitor will also perform subject's validation checks of the study particpant’s questionnaires and may periodically request review of the Investigator's Study File to ensure completeness of documentation in all respects of clinical study conduct.

Upon completion of the study, the study monitor will arrange for a final review of the study files after which the files should be secured for the appropriate time period. The PI or PI designee will receive the study monitor during these on-site visits, cooperate in providing the documents for inspection, and respond to inquiries.

13.2 SOURCE DOCUMENT

The investigator(s)/Institution(s) will permit study-related monitoring, audits by or on behalf of the Sponsor, IRB review and regulatory inspections providing direct access to source data documents. Source documents are original records in which raw data are first recorded. These may be office / clinic / hospital records, charts, diaries, questionnaires and laboratory results, printouts, pharmacy records, care records, completed questionnaires for each study subject. Source documents should be kept in a secure and limited access area. All source documents must be accurate, clear, unambigous, permanent and capable of being audited. They should be made using a permanent form of recording (ink, typing, printing, optical disc etc). They should not be obscured by correcting fluid or have temporary attachements (such as removable self-stick notes). Source documents that are computer generated and stored electronically must be printed (for review by the study monitor), signed and dated by the investigator.

Source data for subjects should indicate date informed consent was signed, participation in clinical protocol number EB-01 and title, evidence that inclusion/exclusion criteria have been met and study execution dates.

13.3 CASE REPORT FORMS (CRFS)

CRFs will be provided to the study site by Envivo Bio Inc. The PI will be responsible for the timeliness, completeness, and accuracy of the information on the CRF. All entries must be legibly recorded. The entry to be corrected is to be crossed out with a single line so that the original entry remains legible. The correction then has to be made right next to the entry and confirmed by date and initials of the person making the correction. Corrections that cannot be made in this fashion have to be explained in a detailed statement (e.g. Data Clarification Form), reference to which has to be documented on the relevant CRF page(s). No one can erase, overwrite, or use correction fluid on the original. The CRF for each subject must be reviewed and signed by the PI. This should be done as soon as possible after each subject completes the study. The PI will make the CRF pages available for review and collection by the designated Envivo Bio Inc.'s representative at each scheduled monitoring visit. A clinical study monitor will review the CRFs for all subjects enrolled and will compare the content versus the source data. The CRF will be on colored paper (for example, yellow). At the end of the study, the CRF will be photocopied with the original colored CRF (yellow) retrieved by the monitor to the Sponsor and the photocopied CRF (white) will remain at the site.

All clinical work conducted under this protocol is subject to GCP rules. This includes an inspection by Envivo Bio Inc. and/or health authority representatives at any time. The PI will agree to the inspection of study-related records by health authority representatives and/or Envivo Bio Inc.

13.4 DATA MANAGEMENT

All protocol-specified data documented on the CRFs as well as the subjects' questionnaires and diaries will be entered into an electronic database by a representative of the Sponsor.

14.0 STUDY ADMINISTRATION

14.1 STUDY PERSONNEL

14.1.1 Site Staff

The Principal Investigator (PI) for this trial will be qualified by education and experience to conduct this clinical trial in accordance with the protocol, ICH Good Clinical Practice (GCP) guidelines and all state, local and federal regulations. The PI is responsible for the conduct of the clincial trial at a trial site.

14.1.2 Sponsor

Below is a list of key individuals and their roles from the Sponsor and designee that will contribute to this study:

Sponsor:	Envivo Bio Inc.
Project Manager:	Dr. Dari Shalon
Study Medical Monitor:	Dr. Shai Friedland

14.2 REQUIRED DOCUMENTS PRIOR TO STUDY INITIATION

Prior to the initiation of the study and enrollment of subjects, the following items must be received by the Sponsor from the investigational site:

- All applicable country-specific regulatory requirements and forms.
- Confidential Disclosure Agreement.
- Signed protocol, amendment(s), Investigator's Brochure and notification(s) page(s).
- Provisions for direct access to source/data documents if necessary for trial-related monitoring, audits, IRB review, and regulatory inspection.
- Curriculum vitae (current < 2 years) and current medical licenses for the Principal Investigator and sub-investigators.
- Signed Clinical Study Agreement and Financial Agreement.
- Appropriate local health authority documentation properly signed and dated by the required investigators.
- Letter of approval from the IRB and local regulatory agency for protocol (identified by protocol title and number), informed consent form (identified by protocol title and number), Subject Information Sheet (if applicable), advertisements (if applicable).
- Copy of the IRB-approved written ICF to be used in the study (that has also been approved by the Sponsor).
- IRB name, address and membership list.
- Financial disclosure information for the principal investigator and sub investigators.
- Name and location of all investigative sites.

Upon satisfactory receipt of all required regulatory documents, Envivo Bio Inc. will arrange that all study material be delivered to the study site. Subject entry should not begin until after the required regulatory documents are confirmed as received and the Investigator Initiation visit has occurred. All personnel expected to be involved in the conduct of the study will undergo orientation to include review of GCP, study protocol, instructions for CRF completion, AE reporting, and overall responsibilities, including those for study file maintenance.

14.3 INVESTIGATOR'S STUDY FILE

Envivo Bio Inc. will provide the site with an Investigator's Study File. This file should be used for all trial-related documents. The site study staff will be responsible for keeping the Investigator's file updated and ensuring that all required documents are filed appropriately. The file will be inspected during monitoring visits and may be audited by the Sponsor and/or regulatory agencies.

The Investigator's Study File will contain the protocol/amendments, Case Report and Query Forms, IRB and governmental approval with correspondence, sample informed consent, study staff curriculum vitae and authorization forms and other appropriate documents or correspondence etc.

14.4 CLINICAL TRIAL SUPPLIES

Clinical trial supplies include, however, not limited to: CRFs, subjects' Daily Diary, saliva collection kit, stool collection kits, and Capscan Devices.

The PI, PI designee or Sponsor will be responsible for the dispensing, inventory, and accountability of all clinical trial supplies. An accurate and timely record of the disposition of all clinical supplies must be maintained. The supplies and inventory record must be made available for inspection by the designated Sponsor's representative upon request. Upon completion or termination of the study the Investigator will return all remaining clinical supplies to Envivo Bio Inc., or Sponsor designee along with a copy of the inventory record and a record of the clinical supplies returned. Under no circumstances will the PI allow the investigational medical device to be used other than as directed by this protocol.

14.5 STUDY REPORTING REQUIREMENT

The Principal Investigator reporting requirements during the study include:

1. Notifying the Envivo Bio Inc. immediately in case of **any Serious Adverse Event and any device-related Adverse Effect.**
2. Inform the IRB about any Serious Adverse Device Effects (SADE’s) as per IRB reporting policy.
3. Reporting to the Sponsor within five (5) working days of a withdrawal of IRB approval at the site.
4. Notifying the IRB and the Sponsor within five (5) working days of the occurrence of any emergency protocol deviations necessary to protect study subjects.

14.6 STUDY COMPLETION

This study is expected to end when all required subjects have been enrolled and the last subject has completed the study and the query resolution has been completed.

A declaration of the end of the clinical trial will be made according to local regulations.

Data and materials that are required before the study can be considered complete and/or terminated are:

- All clinical data and all special test results from screening through the end of the follow-up period

- CRFs (including correction forms) properly completed by appropriate study personnel and signed by the PI
- Statement of outcome for each serious adverse event reported
- Copies of approved protocol amendments by IRB committee as well as relevant health authority approval/notification (if applicable)

14.7 FINAL REPORT

The PI will complete a report notifying the IRB of the conclusion of the clinical study.

The final report sent to IRB will also be sent to the Sponsor and, along with the completed CRFs, will constitute the final summary to the Sponsor, thereby fulfilling the PI's regulatory responsibility.

14.8 RETENTION OF STUDY RECORDS

The PI will retain copies of the approved protocol, completed CRFs, informed consent documents, relevant source documents, and all other supporting documentation related to the project in a secured and safe facility for one of the following periods based on notification from the Sponsor:

- A period of at least two years from last device registration and notification from the Sponsor
- Or a period of at least two years after discontinuation of clinical development of the investigational product as confirmed by the Sponsor
- Or longer if required by local regulations.

These files must be made available for inspection upon reasonable request by authorized representatives of Envivo Bio Inc., or the corresponding regulatory agencies of the relevant countries.

Envivo Bio Inc. will provide the PI with information concerning the current status of the investigational medical device as it relates to the investigator's obligation for the retention of study records. The PI should contact Envivo Bio Inc. prior to disposing of any such records and to provide written notification to the Sponsor of any change in the location, disposition, or custody of the study files. Envivo Bio Inc. will arrange for continued storage of all records, if necessary.

14.9 CONFIDENTIALITY AND PUBLICATION OF STUDY RESULTS

All unpublished information given to the PI by the Sponsor shall be kept confidential. Results of this study will not be submitted for presentation or publication or disclosed to a third party without the prior written permission of Envivo Bio Inc. The publication and communication rights are found in the Investigator agreement. The Sponsor requires that manuscripts or abstracts containing information about or data derived from this study be submitted to the Sponsor at least 30 days prior to submission to the journal or conference presentation, to safeguard against disclosure of confidential information.

When the Sponsor generates reports for presentations to regulatory agencies, an endorsement of the final report may be sought from the investigators who have contributed significantly to the study.

No patent application based on the results of this study shall be made by the Investigator, and no assistance shall be given to any third party for such an application, without the written authorization of the Sponsor.

REFERENCES

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[iv] Wlodarska M. et. al. An integrative view of microbiome-host interactions in inflammatory bowel diseases, Cell Host Microbe. 2015 May 13; 17(5): 577–591.

[v] Li G, Yang et al. Diversity of duodenal and rectal microbiota in biopsy tissues and luminal contents in healthy volunteers. J Micro Biotech. 2015;25(7):1136–45.

Appendix A

Subject initials: _____ Subject no: _____ Today's Date: _____

(Date notation 01 May 2022**)**

ENVIVO BIO DAILY DIARY

Instructions: Please complete **all** questions below.

1) Circle CapScan Device number swallowed today and time of swallowing:

Capsule number	1	2	3	4	5	6
Time swallowed						

(Time notation 9:15 am, 5:30 pm)

2) Time sulfasalazine taken:_____ Time blood sampled:_____ and _____

3) What did you eat and drink today:

Food	Time Finished Eating

4) When did you have bowel movements today and did you retrieve a CapScan Device in the toilet:

Bowel movement number	1	2	3	4	5
Time					
Retrieved device (Yes or No)					