Clinical Study Protocol

IND Number	16514
Test Product	Enterotoxigenic *E. coli* (ETEC) strain E24377A
Study code:	OEV-131
Protocol Version and date	Final 3.0, 23-FEB-2024

STUDY TITLE

Pre-Study of wild type Enterotoxigenic *E. coli* (ETEC) Strain for Verification of a planned Challenge Dose

Test product and dosage	Approximately 4 x 10^9 cfu of Enterotoxigenic *E. coli* (ETEC) strain E24377A
Comparator product and dosage	Not applicable
Duration of treatment	Single administration of challenge dose
Medical Advisor (Sponsor signatory)	[redacted] Scandinavian Biopharma Holding AB, Solna, Sweden [redacted]
Principal Investigator	[redacted] Johns Hopkins Bloomberg School of Public Health [redacted]

The following amendments have been made to the Final Clinical Study Protocol version 2.0:

Amendment No.	**Date of Amendment**	**Revised protocol version** *(if applicable)*
1.0	23-Feb-2024	Version 3.0, dated 23-FEB-2024

2 PROTOCOL SYNOPSIS

Study Title: Pre-Study of wild type Enterotoxigenic *E. coli* (ETEC) Strain for Verification of a planned Challenge Dose	
Study code: OEV-131	**IND Number: 16514**
Study period Estimated date of first subject screened: Q1 2024 Estimated date of last subject completed: Q3 2024	**Phase of development: 1**
Principal Investigator [redacted] M.D. [redacted]	
Study design An open label study with the aim to estimate the incidence of moderate and severe diarrhea among participants challenged with [redacted] of the *E. coli* E24377A strain. This dose is planned to be used in a subsequent challenge study of the efficacy of an [redacted] (ETVAX®).	
Objectives **Primary objective** To evaluate the moderate and severe diarrhea attack rate after challenge with wild type enterotoxigenic *E. coli* (ETEC) strain E24377A **Primary endpoint** Moderate and severe diarrhea, as defined by ≥ 4 grade 3-5 stools **or** > 400 grams of grade 3-5 stools passed within a rolling 24-hour period, deemed attributable to ETEC. Diarrhea episodes or ETEC symptoms beginning any time after challenge through 120 hours post-challenge may contribute toward the primary endpoint and will be followed to resolution. The end of a diarrheal episode occurs when a subject does not pass any grade 3-5 stool within 24 hours. An adjudication committee will be used to judge if the diarrhea is attributable to ETEC and determine if a subject meets the primary endpoint by considering individual data as described in the adjudication charter. **Secondary objectives** • To further evaluate the clinical features of the challenge with wild type enterotoxigenic *E. coli* (ETEC) strain E24377A • To evaluate the safety of the challenge with wild type enterotoxigenic *E. coli* (ETEC) strain E24377A	

Number of subjects planned

Up to 30 healthy subjects are planned to be enrolled in the study. Up to 32 subjects will be invited to the in-patient unit to ensure up to 30 are available for challenge.

Diagnosis and eligibility criteria

Inclusion criteria:

1. Healthy adults between 18 and 50 years of age, inclusive, at the time of signing the informed consent.
2. General good health, without clinically significant medical history, physical examination findings or clinical laboratory abnormalities per clinical judgment of PI.
3. Negative pregnancy test at screening and prior to challenge for people of childbearing potential. People of childbearing potential must agree to use an efficacious hormonal or barrier method of birth control during the study. Abstinence is acceptable. People of childbearing potential unable to bear children must have this documented (e.g. tubal ligation or hysterectomy) or must have negative pregnancy tests at screening and prior to challenge.
4. Willingness to participate in the study after all aspects of the protocol have been explained and written informed consent obtained.
5. Completion of a training session and demonstrated comprehension of the protocol procedures and knowledge of ETEC associated illness by passing a written examination (70% pass score).
6. Availability for the study duration, including planned follow-up visit/contact.

Exclusion criteria:

1. Presence of a significant medical or psychiatric condition which in the opinion of the investigator precludes participation in the study, including gastrointestinal disease (gastritis, irritable bowel disease as suggested by Rome III criteria or medical diagnosis, inflammatory bowel disease). Some medical conditions which are adequately treated and stable would not preclude entry into the study. These conditions might include stable asthma controlled with inhalers or mild hypertension stably controlled.
2. Significant abnormalities in screening haematology or serum chemistry as determined by PI.
3. Presence in the serum of HIV antibody, HBsAg, or HCV antibody with confirmation of infection (e.g. by HCV PCR).
4. Evidence of IgA deficiency (serum IgA < 7 mg/dl or limit of detection of assay).
5. Evidence of current alcohol or drug dependence.
6. Subjects whose Body Mass Index (BMI) is less than 19.0 or greater than 37.0 (kg/m^2).
7. Recent vaccination or receipt of an investigational product (within 30 days before challenge) or intended vaccination or receipt of investigational products until 60 days after challenge, with the exception of licensed vaccine for influenza or SARS-CoV-2 vaccination that may be given up to 7 days prior to challenge.
8. Positive test for SARS-CoV-2 at arrival to the unit on the day of in-patient admission.
9. Intention to donate blood or blood products within one month following the completion of study participation (note: The Red Cross will not allow blood donations for 1 year following participation in an investigational research study).
10. Any other criteria which, in the investigator's opinion, would compromise the ability of the subject to participate in the study, the safety of the study, or the results of the study.
11. Abnormal stool pattern (fewer than 3 per week or more than 3 per day).
12. Regular (≥ weekly) use of laxatives, antacids, or other agents to lower stomach acidity.
13. Use of any medication known to affect the immune function (e.g., corticosteroids and others) within 30 days preceding the challenge or planned use during the active study period. Use of inhaled or topical steroids may be permitted per PI discretion.
14. History of microbiologically confirmed ETEC infection in the last 3 years.

15. Occupational handling of ETEC currently, or in the past 3 years.
16. Travel to countries where ETEC infection is endemic (most of the developing world) within two years prior to dosing OR visit for > two months in ETEC endemic countries during the last 10 years, OR planned travel to endemic countries prior to study day 180.
17. Vaccination for or ingestion of ETEC, cholera, or LT toxin within 5 years prior to dosing.
18. Use of antibiotics during the 14 days before challenge dosing or proton pump inhibitors, H2 blockers or antacids within 48 hours prior to challenge dosing.
19. History of diarrhea in the 7 days prior to challenge (outpatient diarrhea is defined as ≥ 3 unformed (grade 3 or greater) loose stools in 24 hours).
20. Known allergy to two of the three following antibiotics: ciprofloxacin, amoxicillin, and/or azithromycin.

Methodology

The study will consist of three study periods: screening, in-patient period and out-patient follow-up. Subjects will be evaluated for study eligibility during the screening period. After provision of written informed consent, subject eligibility for study entry will be assessed. Subjects who have been assessed eligible for the study, based on screening evaluations, will be admitted to the in-patient unit on Day -1, the day before E24377A administration, for study orientation and to be monitored for any signs and symptoms of illness. At baseline (Day 1), subjects who will remain eligible and willing to participate will be enrolled in the study and receive a single administration of E24377A. Subjects may remain in the in-patient unit until Day 9 (routine discharge is scheduled for 8 days after challenge dependent upon 2 consecutive negative stool culture results). During in-patient period, subjects will be examined on a daily basis for signs and symptoms of ETEC illness. Vital signs will be measured at least 3 times daily starting on Day 1, and postural blood pressure and pulse will be measured as needed. All stools will be collected and evaluated, weighed, and graded by the study staff. Up to 3 stools and/or rectal swabs each day will be cultured.

If the subject develops any symptom suggestive of diarrhea, they will be encouraged to drink liquids. If a subject vomits or is unable to consume an adequate volume of liquids, then intravenous (IV) fluids will be administered.

In order to reduce the risk of secondary infection after discharge, all subjects will receive a 3-day course of antibiotics. The subject will begin a 3-day course of ciprofloxacin 500 mg by mouth twice daily (or azithromycin 500 mg by mouth daily for three days, or amoxicillin 500 mg by mouth three times daily for three days if the subject was allergic to ciprofloxacin) on the morning of Day 6, or sooner if the subject has met the criteria for early antibiotic treatment (see Section 9.4.8.2.)

Subjects will be discharged from the in-patient unit if determined eligible for discharge. Subjects who meet the criteria for early antibiotic treatment, may be discharged from the in-patient unit earlier than anticipated upon meeting discharge criteria. Discharge criteria include at least 2 stools negative for the challenge organism, having taken at least 2 doses of antibiotics, and have improvement or resolution of symptoms.

After discharge from the in-patient unit, all subjects will attend the scheduled follow-up visit/contact on an out-patient basis on Day 29 (physical visit) and Day 180 (phone).

Test Product, dosage and mode of administration

Each subject will drink 120 mL of sodium bicarbonate buffer (in order to neutralize gastric acidity) followed one to two minutes later with 30 mL of sodium bicarbonate buffer containing approximately 4 x10^9 cfu of the challenge strain E24377A. All subjects will have fasted for approximately 90 minutes before challenge and for approximately 90 minutes after challenge.
Reference Therapy, dosage and mode of administration *(if applicable)* Not applicable in this study.
Duration of treatment All subjects will receive a single administration of E24377 at baseline (Day 1). The study period includes a 5-10 day in-patient period that will be followed by a 3-week outpatient follow-up period and there will be a final contact (phone call) approximately 6 months after challenge.
Duration of subjects' involvement in the study The total duration of any subject's participation in the study will be up to around 35 weeks including a screening period of up to 60 days, a 5-10 day in-patient period and 26 weeks (approximately 6 months) follow-up after challenge.
Efficacy assessments Clinical indicators will include the incidence, severity, and time to onset of diarrhea, the incidence and duration of positive stool cultures, the incidence of moderate and severe ETEC illness, evaluation of the number and weight of grade 3-5 stools, and the incidence of malaise, loss of appetite, headache, chills, fever, nausea, abdominal pain, abdominal cramps, myalgia, arthralgia, urgency of defecation, vomiting and lightheadedness. Stool grading criteria: grade 1 = firm, formed; grade 2 = soft but still formed; grade 3 = thick liquid; grade 4 = thin liquid; grade 5 = clear or translucent, watery.
Safety assessments Safety evaluations will include documentation of adverse events and key vital sign measurements.
Statistical methods No formal statistical testing is planned for this study. The proportion of subjects with moderate and severe diarrhea will be presented with 95% confidence intervals.

3 TABLE OF CONTENTS

4 LIST OF ABBREVIATIONS

Abbreviation or term	Explanation
ADL	Activities of Daily Living
AE	Adverse Event
AESI	Adverse Event of Special Interest
BID	Twice daily
BMI	Body Mass Index
CF	Colonization Factor
CFA	Colonization Factor Antigen
cfu	Colony-forming units
CHIM	Controlled Human Infection Model
CI	Confidence Interval
CIR	Center for Immunization Research
COVID-19	Coronavirus disease 2019
CRA	Clinical Research Associate
CRO	Clinical Research Organization
CS	Coli surface antigen
CSP	Clinical Study Protocol
CSR	Clinical Study Report
CT	Cholera Toxin
CTA	Clinical Trial Agreement
cGMP	Current Good Manufacturing Practice
CV	Curriculum Vitae
[redacted]	[redacted]
DMP	Data Management Plan
E. coli	Escherichia coli
eCRF	Electronic Case Report Form
ELISA	Enzyme-linked Immunosorbent Assay
ETEC	Enterotoxigenic *Escherichia coli*
FAS	Full analysis set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
hpf	High-power field
ICF	Informed Consent Form
ICH	International Council for Harmonization
IDS	Investigational Drug Service
IgA	Immunoglobulin A
IgG	Immunoglobulin G

Abbreviation or term	Explanation
IND	Investigational New Drug
IRB	Institutional Review Board
ISF	Investigator Site File
IV	Intravenous
LCTB*A*	Recombinant hybrid protein between B-subunit Heat Labile Toxin from *E. coli* and Cholera Toxin B subunit
LMIC	Low- and middle-income countries
LPS	Lipopolysaccharide
LT	Heat-labile toxin
LTB	Heat-labile toxin B subunit
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Operations
OEV	Oral ETEC Vaccine
PATH	Program for Appropriate Technology in Health
PCR	Polymerase Chain Reaction
PDVAC	WHO´s Product Development for Vaccines Advisory Committee
PI	Principal Investigator
PPAS	Per protocol analysis set
PT	Preferred term
RBC	Red blood cell
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SBH	Scandinavian Biopharma Holding AB
SDV	Source Data Verification
SOC	System Organ Class
SSP	Study Specific Procedure
ST	Heat Stable Toxin
STh, STp	Subtypes of heat stable toxins
SUSAR	Suspected Unexpected Serious Adverse Reaction
TD	Travelers' Diarrhea
US	United States
USP	United States Pharmacopeia
WBC	White blood cell
WHO	World Health Organization
WRAIR	Walter Reed Army Institute of Research

Abbreviation or term Explanation

5 ETHICS AND REGULATORY REQUIREMENTS

5.1 Ethical conduct of the study

The study will be performed in accordance with ethical principles that have their origin in the Declaration of Helsinki and are consistent with ICH/Good Clinical Practice (GCP) E6(R2), and applicable local regulatory requirements.

The Declaration of Helsinki is included as Appendix 13.2 to the Protocol.

5.2 Ethics and regulatory review

Before enrollment of subjects into this study, the clinical study protocol, the subject information and informed consent form, any other written information to be provided to the subjects and any advertisements to be used for recruitment of study subjects will be reviewed and approved by the IRB and applicable regulatory authority in accordance with local requirements.

5.3 Subject information and consent

It is the responsibility of the Principal Investigator to give each potential study subject adequate verbal and written information regarding the objectives and the procedures of the study as well as any risks or inconvenience involved before including the subject in the study. Informed consent is an ongoing process which includes the informed consent document. Prospective subjects will receive an oral or powerpoint presentation of the study. The subject must be informed about the right to withdraw from the study at any time. The subject should be allowed sufficient time to ask questions, have his/her questions answered, and given time for consideration of the study. Any question that cannot be answered will be referred to the PI. The participant will be allowed to take the consent document home to consider and discuss it with others. The participant should understand that the study products are investigational and are not licensed by the FDA for commercial use but are permitted to be used in this clinical research.

To document subjects' understanding of informed consent, immediately before the consent is signed, the person obtaining consent will administer a brief quiz or comprehension test. Incorrect answers will be discussed with subjects to reinforce the consent and subjects will be given one additional opportunity to take the test. A final acceptable test score is 70% or more answered correctly. Subjects failing after 2 attempts will not be eligible for study enrollment. Before subjects participate in the study, consent forms will be signed and dated by subjects as well as by the PI or designee. Subjects will receive copies of the signed consent prior to participation. As part of the consent process, subjects will also be asked to read and sign the Correct Hand Washing Procedure, Alternate Agreement, Inpatient Unit Guidelines, HIPAA Medical Record Release Form, HIV Test Counseling, COVID Vaccination Form and/or any other relevant IRB-approved forms and given an opportunity to ask questions.

Documentation of the discussion and the date of informed consent must be recorded in the source documentation and in the Case Report Form (CRF). The signed ICF should be filed by the Investigator for possible future audits and/or inspections.

5.4 Subject data protection

Investigator must create a Subject Identification List which includes sufficient information to link records, i.e. the CRF and clinical records. This list should be preserved for possible future inspections/audits but should not be made available to SBH or representatives except for monitoring, inspection or auditing purposes.

All study-related information will be stored securely at the study site. All subject information will be stored in locked areas with access limited to study staff. Individual subject information obtained as a result of this study is considered confidential and disclosure to third parties other than those cited below is prohibited without the written signed request of the subject. Subject confidentiality will be further ensured by utilizing subject identification codes. Computer entry of data will be done by coded number and all local databases will be secured with password-protected access systems.

The potential study subject must be informed that by signing the ICF he/she approves that authorized representatives as shown below may have direct access to his/her medical records:

- Audit and Compliance Officers and Legal Counsel
- The U.S. Food and Drug Administration (FDA) and other similar regulatory agencies
- The study's Sponsor and authorized representatives
- Study monitors, Medical Monitors
- Governmental agencies to which HIV and hepatitis testing must be reported
- The Johns Hopkins University Bloomberg School of Public Health
- The Office of Human and Animal Research Oversight (OHARO)

Subjects' study information will not be released to any other party without additional written permission of the subject.

The ICF describes the volunteer data that will be recorded, collected and processed. In accordance with applicable regulatory requirements for data privacy and protection, the data will not identify any persons taking part in the study.

6 INVESTIGATOR(S) AND STUDY ADMINISTRATIVE STRUCTURE

This clinical study is sponsored by the Swedish company Scandinavian Biopharma Holding AB. The study will be conducted at the Center for Immunization Research, Johns Hopkins Bloomberg School of Public Health in Baltimore, MD.

Clinical Monitoring, Data Management, biostatistics and safety reporting will be provided by the [redacted].

Key roles are presented below.

Sponsor´s Medical Advisor	[redacted] Scandinavian Biopharma Holding AB

	Industrivägen 1 SE-171 48 Solna, Sweden Email: [redacted]
Principal Investigator:	[redacted] MD
Sub-Investigator:	[redacted]
Sub-Investigator	[redacted]
Subinvestigators	[redacted]
Epidemiology	[redacted]
Clinical Monitoring, Safety Reporting, Data Management, and Statistics	[redacted]
Research Laboratories:	[redacted]
Clinical Laboratories:	[redacted]

Signatures required should be provided in appendix 13.1.

7 INTRODUCTION

7.1 Project background

Enterotoxigenic *Escherichia coli* (ETEC) bacteria cause considerable physical suffering and malnutrition due to repeated bouts of illness in children in low- and middle-income countries (LMIC). Diarrhea is the second-leading cause of death among children <5 years of age worldwide. The actual mortality numbers are difficult to estimate; global annual incidences of nearly 1.7 billion cases of childhood diarrheal disease with approximately 500 000 – 700 000 deaths in children <5 years of age have been presented [1, 2]. The relative importance of ETEC versus other diarrheal pathogens for the total burden of diarrheal disease is debated [2, 3]. However, the significant morbidity caused by ETEC in young children is undisputed, and it is also clear that repeated ETEC episodes have significant impact on growth and mental development [4, 5, 6]. WHO's PDVAC (Product Development for Vaccines Committee) has 2020 proclaimed that an ETEC vaccine is a high priority for LMICs [4, 5]. A vaccine against ETEC would have a significant impact on travelers as well. Prior to the global SARS-CoV-2 pandemic, 500-600 million people traveled annually to LMICs [7]. Traveling to LMIC involves a risk of travel-related diseases and at least 35 million travelers per year are affected by travelers' diarrhea (TD) [8, 9]. ETEC is the major cause of TD, responsible for approximately 15 million annual cases [8, 9].

ETEC spreads through the fecal-oral route. Infection occurs when a person ingests food or liquid contaminated with ETEC bacteria. Human waste (e.g. feces) is the ultimate source of ETEC contamination. ETEC can adhere to the mucosal epithelial cells lining the small intestine using colonization factors (CF, also called coli surface antigens, CS) [6]. Once attached to the mucosal surface, the bacterium starts to produce a heat labile (LT) and/or a heat stable toxin (ST)

triggering diarrhea. The LT toxin is highly homologous to the cholera toxin (CT) and causes diarrhea via a highly similar mechanisms [6].

ETVAX® is [redacted] ETVAX® is currently in late phase development and has been studied in several clinical phase 1 and 2b studies. Successfully completed clinical studies have shown that the vaccine is safe and immunogenic, giving rise to mucosal IgA responses to all vaccine CFs and to LTB, both in children living in endemic areas, as well as in Western healthy adults [11, 12, 13, 14, 15]. Our previous results also demonstrate that the vaccine gives rise to memory B cell responses that can be detected until at least 2 years after vaccination and that such long-term immunity may be reflected by the circulation of activated follicular helper T cells early after immunization [13, 16].

The product formulation of ETVAX® [redacted] has been established. In the [redacted] are mixed with the buffer salts and stabilizing excipients in a sachet. In preparation of the partially dried formulation, the content of the [redacted] is dissolved in 150 ml of tap water, followed by the addition of the vaccine vial content (inactivated bacteria). The new product formulation is thus very simple to prepare and suitable for use in the field. A two-armed, non-inferiority, immunogenicity and safety study in 280 adult volunteers at one study site in Sweden comparing the two formulations, has recently been completed. The noninferiority margin was set to -15% (on an absolute scale). The study demonstrated that the new partially dried formulation is noninferior in eliciting an immune response to LTB compared to the old wet formulation. The seroconversion rate was 83% and 85% in the partially dried formulation and wet formulation respectively with a 95% confidence interval for the difference between the seroconversion rates of (-11%, 7%). The results show that the new partially dried vaccine formulation was safe and well tolerated [17].

A pivotal phase 3 study to demonstrate efficacy to support licensure for the travelers' diarrhea indication is currently planned using a Controlled Human Infection Model (CHIM). In preparation for the phase 3 CHIM study, this pre-study will be performed to estimate the incidence of moderate and severe diarrhea among participants challenged with $4x10^9$ cfu of the ETEC strain E24377A. [redacted] This CHIM study is designed to demonstrate the protective efficacy of ETVAX® and to collect additional safety and immunogenicity data.

7.2 Challenge strain E24377A

The ETEC strain E24377A expresses the CS1 and CS3, as well as the STh (a subtype of the ST toxin) and LT enterotoxins. The strain also expresses the O139 Lipopolysaccharide (LPS) antigen and H28 flagella. E24377A was, in the early 1980s, isolated from a traveler returning from Egypt with an ETEC diarrheal illness [18], and it shares the CS3 and LT antigens with one of the *E. coli* strains in the ETVAX® vaccine but is of a different O and H antigen type. The CS1+CS3 CF combination of virulence factors are well-represented in field isolates and contribute to virulence, infectivity, and disease severity throughout the world. They are seen in ETEC strains causing disease in both adults and children globally [19]. The ETEC strain E24377A has been fully DNA sequenced [20, 21], including a comparative genomic analysis of *E. coli* commensal and pathogenic isolates [20].

The E24377A (lot 0807) challenge strain was manufactured under current Good Manufacturing practice (cGMP) on August 31, 2000 and released 17 April, 2001 for use in a CHIM study.

For this pre-study and the planned CHIM study involving the ETVAX® vaccine, the E24377A ETEC strain is considered to be the best suited as (a) there is a fair amount of experience with E24377A (third most commonly used challenge strain [22], (b) the disease severity score is predominantly moderate [23] and (c) the time to diarrhea is with a median of 25 hours post-challenge, also intermediate compared to common ETEC challenge strains (e.g. 47 hours for H10407 and 13 hours for B7A) and, therefore well-suited for a 5-10 day in-house challenge period [23]. The E24377A strain has also been used in prior vaccination-challenge studies [18, 24, 25, 26, 27, 28], see Section 9.2.

The 0807 GMP lot of the ETEC E24377A strain has been used in previous CHIM studies to assess the efficacy of other candidate ETEC vaccines [18, 24]. Challenge doses ranging from $6x10^8$ to $4x10^9$ were used with overall diarrheal rates of approximately 80% and with moderate-to-severe diarrhea rates of 60-81%. This planned pre-study will be conducted with the aim to estimate the incidence of moderate and severe diarrhea among participants challenged with $4x10^9$ cfu of the E24377A strain.

7.3 Risk/benefit assessment

7.3.1 Benefits

Study subjects will have no direct benefit from study participation. However, subjects will receive a free health check-up and there is a potential future societal benefit should the outcome of this study lead to a subsequent vaccination and challenge study resulting in a vaccine able to prevent TD caused by ETEC.

7.3.2 Risks

Risks Associated with ETEC Infection: The main risk is also the primary objective in this study: diarrhea caused by ETEC. Infection with ETEC can cause profuse watery diarrhea, abdominal cramping, abdominal pain, malaise, headache, urgency of defecation, lightheadedness and/or dehydration. Fever, nausea with or without vomiting, chills, loss of appetite, muscle aches and bloating can also occur but are less common. Illness develops 1-3 days after exposure and usually lasts 3-4 days [29] without treatment, but generally improves within 24 hours of antibiotic therapy.

In order to reduce the risk of secondary infection after discharge, all subjects in the study will receive a 3-day course of antibiotics starting no later than Day 6. Ciprofloxacin is the first-line antibiotic treatment for all subjects. If a subject is unable or unwilling to take ciprofloxacin, azithromycin or amoxicillin will be used instead. The risk of diarrhea complications will be minimized by a conservative approach to timing of antibiotic administration, well within an interval that has been shown to be efficacious, as well as daily clinical monitoring. Stool output will be closely monitored.

Risks Associated with Post-Infectious Irritable Bowel Syndrome (PI-IBS): Recent studies also suggest an increased risk of post-infectious irritable bowel syndrome (PI-IBS) following bacterial enteritis, and infection with ETEC has been found to be associated with these sequelae. A long duration (>2 weeks) of TD has been shown to be a risk factor for development of PI-IBS [30]. Prompt treatment of the ETEC infection, as done in the present study, cuts the duration of

any ETEC illness short and thus most likely eliminates the risk of PI-IBS. Symptoms of PI-IBS will be screened for with the Functional Bowel Disorder Survey (FBS) administered on study day 180. A study provider will review each FBS. Any participant identified as having a risk for PI-IBS will be referred for follow-up care. PI-IBS may be detected in 3-17% of people who experience travelers diarrhea (TD) [9], however there have been no documented cases of PI-IBS in enteric challenge study participants [31].

Risks Associated with Antibiotics: Therapeutic antibiotics for use in this study are licensed, approved medications that have been used extensively, and shown to be very safe with only rare side effects. The most commonly reported side effects for ciprofloxacin are gastrointestinal symptoms (nausea, vomiting, and diarrhea) in as many as 5 persons in 100. Other reported symptoms in less than 1 person in 100 include rash, dizziness, and headache. Rarely, allergic reactions to these medications have been observed. Ciprofloxacin is not recommended for use in pregnancy due to concerns of joint damage to the unborn child (based on studies in young animals). Pregnancy is exclusionary for study participation and is documented through testing prior to study interventions and provided discussion on methods to prevent pregnancy during study. Fluoroquinolones, including ciprofloxacin, are associated with an increased risk of tendonitis and tendon rupture in all ages. The risk of developing fluoroquinolone-associated tendonitis and tendon rupture is further increased in older patients, usually over 60 years of age, in patients taking corticosteroid drugs, and in patients with kidney, heart, or lung transplants, all of whom are excluded from this study. *Clostridium difficile*-associated diarrhea (CDAD/ pseudomembranous colitis) has been reported with use of nearly all antibacterial agents.

Subjects will not be discharged until they are well and have had at least two consecutive stool cultures negative for the challenge strain. Both stool cultures may be from the same day. Approximately 6 months after challenge, all subjects will have a follow-up contact by telephone to inquire about the occurrence of any new chronic illnesses or serious adverse events.

Risks Associated with SARS-CoV-2 Exposure and Risk Mitigation: The risk that the COVID-19 pandemic will influence the possibility to perform the study according to plan, or influence the results of the study, will be continuously assessed by the study team. Since the subjects are planned to be enrolled in Q1 2024, when a majority of the adult US population will have been vaccinated or infected with SARS-CoV-2, the risk that the pandemic will have major influence on the study is deemed to be small.

It is the intent of the Center for Immunization Research (CIR) to mitigate risk and provide a safe environment for participants while they are inpatient on the unit during the study. The CIR will educate and encourage all potential study volunteers to be fully vaccinated with one of the COVID licensed/EUA vaccines prior to enrolling in this study. Volunteers will not be required to be vaccinated against SARS-CoV-2, however, vaccinated volunteers will be prioritized for enrollment.

The CIR will follow recommendations from the CDC when implementing clinical trials. The CIR will also follow institutional guidelines and policies. Current recommendations include vaccination with one of the licensed/EUA vaccines for all adults.

Volunteers will be educated on the risks of not being vaccinated. They will sign a disclaimer regarding vaccination status and possible exposure to the virus. Volunteers will be strongly encouraged to wear a mask and maintain social distance from others.

Depending on the level of COVID illness in the community and current CDC and JHU guidelines, volunteers may be asked to obtain a COVID-19 test 1-5 days prior to admission. All volunteers will be screened for symptoms and tested on arrival to the unit on the day of inpatient admission. Potential volunteers who test positive for SARS-CoV-2 will not be eligible for enrollment.

Risks Associated with Venipuncture and IV fluid administration: Blood sampling of study participants may cause pain at the time of sample collection and may result in the development of bruising, bleeding, lightheadedness, syncope (rarely) and infection (rarely). Intravenous catheters may additionally infiltrate and cause swelling and/or discomfort.

Risks Associated with Stool Collection: There are no risks associated with the collection of stool samples. However, participants may be asked to provide up to three rectal swabs per inpatient day as needed. Risks of using rectal swabs could include mild discomfort, embarrassment, and (very rarely) bleeding or irritation.

Risks Associated with Transmission of Challenge strain: This *ETEC* strain has the potential for risk to both the environment and to the research personnel; however, the risk to the environment in regard to potential transmission outside of the CIR facility is low. The risk to the environment will be reduced by:

- ensuring that all human waste products from inpatients are disinfected with bleach prior to disposal,
- emphasizing the importance of handwashing for participants and staff,
- ensuring proper disposal/cleaning of linen,
- ensuring participants will not be discharged until they are no longer shedding the challenge strain, as per procedures outlined in the protocol.

Risks Associated with Isolation: Volunteers may feel bored or anxious about not being able to leave the unit or being separated from family, friends, and community while they are on the unit. The unit will be staffed 24/7 with medical staff and overnight with security staff. There will be planned activities in which the subjects can participate. There are big screen TVs throughout the unit as well as games, and a kitchen and dining area.

Risks Associated with Laboratory Results: There may be physical, psychological, and social risks if participants test positive for hepatitis B, hepatitis C, and/or HIV. Participants testing positive will be counseled and referred for treatment.

Risks Associated with Breach of Confidentiality: All data and medical information obtained about participants will be considered privileged and held in confidence. A breach of confidentiality in which private health information is made public is possible. Participants will not be identified by name in any published report/presentation of the results. The Sponsor, their delegates, and the FDA may inspect the records of this research as part of their responsibility to oversee research and ensure protection of participants. Study results and data may be published in scientific/medical journals; the identity of individual participants will not be disclosed.

8 STUDY OBJECTIVES AND ENDPOINTS

8.1 Primary objective

To evaluate the moderate and severe diarrhea attack rate after challenge with wild type enterotoxigenic *E. coli* (ETEC) strain E24377A

8.1.1 Primary endpoint

Moderate and severe diarrhea, as defined by ≥ 4 grade 3-5 stools **or** > 400 grams of grade 3-5 stools passed within a rolling 24-hour period, deemed attributable to ETEC.

Diarrhea episodes or ETEC symptoms beginning any time after challenge through 120 hours post-challenge may contribute toward the primary endpoint and will be followed to resolution. The end of a diarrheal episode occurs when a subject does not pass any grade 3-5 stool within 24 hours. An adjudication committee will be used to judge if the diarrhea is attributable to ETEC and determine if a subject meets the primary endpoint by considering individual data as described in the adjudication charter.

Stool grading criteria: grade 1 = firm, formed; grade 2 = soft but still formed; grade 3 = thick liquid; grade 4 = thin liquid; grade 5 = clear or translucent, watery

8.2 Secondary objectives

- To further evaluate the clinical features of the challenge with wild type ETEC strain E24377A
- To evaluate the safety of the challenge with wild type ETEC strain E24377A

8.2.1 Secondary Clinical endpoints

- Severe diarrhea defined as ≥6 grade 3-5 stools or >800g of grade 3-5 stools passed within a rolling 24-hour period.
- ETEC disease severity: 3-component disease score (total score) utilizing objective signs, subjective symptoms and stool output [31].
- Incidence of diarrhea (≥2 grade 3-5 stools in a 24-hour period) of any severity.
- Total weight of grade 3-5 stools passed per subject over the 120-hour observation period.
- Number of grade 3-5 stools per subject over the 120-hour observation period.
- Occurrence of other ETEC Disease-specific events (malaise, loss of appetite, headache, chills, fever, nausea, abdominal pain, abdominal cramps, myalgia, arthralgia, urgency of defecation, vomiting and lightheadedness).
- Self-assessment of ETEC illness impact on daily activity at Day 6.

- Time (relative to challenge) to the first grade 3-5 stool of the first diarrhea episode.
- Time (relative to challenge) to meeting the primary endpoint (time of the stool that meets the criteria for at least moderate diarrhea).
- Requirement of early (prior to Day 6) antibiotic treatment.
- Requirement of IV fluids.
- Maximum 24-hour grade 3-5 stool output (weight).
- Maximum number (24-hour) of grade 3-5 stools.

8.2.2 Secondary Microbiology endpoint

- Qualitative shedding of the E24377A challenge strain post-challenge (positive or negative)

8.2.3 Secondary Safety endpoints

- Adverse events leading to study withdrawal
- Serious adverse events (SAEs) occurring during the 6 months of the study (from the time of challenge)

8.3 Exploratory Objective(s)

8.3.1 Exploratory immunogenicity objective

To evaluate immunogenicity of the challenge with wild type enterotoxigenic *E. coli* (ETEC) strain E24377A

8.3.1.1 Exploratory immunogenicity endpoints that may be conducted

- Serum IgA and IgG antibody seroconversion (≥2-fold increase over baseline to challenge strain specific antigens LT, CS1, CS3 in serum) from baseline prior to challenge and post-challenge. In addition, ≥4-fold increase may be explored.
- Increases in serum IgA measurements utilizing Antibody in the Lymphocyte Supernatant (ALS) assay in response to LT, CS1, and CS3.
- Increases in Memory B cell measurements utilizing Antibody in the Lymphocyte Supernatant (ALS) assay.
- Whole blood for transciptomics.
- Increases in Fecal IgA levels to LT, CS1, CS3 from baseline prior to challenge and post-challenge.
- Biomarker assessments to measure intestinal inflammation in stool (e.g. calprotectin, neopterin, myeloperoxidase).
- Biomarker assessments to measure systemic inflammation in serum (e.g. C-reactive protein, Intestinal Fatty Acid binding protein).
- Fecal microbiome.assessment.

Specific collection days will be detailed in the Manual of Procedures.

8.3.2 Exploratory microbiology objective

To evaluate the level of quantitative fecal shedding (colony forming units per gram of stool) of the E24377A challenge strain post-challenge

8.3.2.1 Exploratory microbiology endpoints

- Number of colony forming units (cfu) per gram of stool of the E24377A challenge strain on Days 2 and 4 after challenge

9 INVESTIGATIONAL PLAN

This is an open label study with the aim to estimate the incidence of moderate and severe diarrhea among participants challenged with $4x10^9$ cfu of the *E. coli* E24377A strain. [redacted]

The study will be conducted at one site; the Center for Immunization Research, Johns Hopkins Bloomberg School of Public Health in Baltimore, Maryland, United States.

Up to 30 (minimum 25) subjects will be enrolled and challenged with approximately $4x10^9$ cfu of the *E. coli* E24377A strain.

Overall description of study design

The study will consist of three study periods: screening, inpatient period and outpatient follow-up. Subjects will be evaluated for study eligibility during the screening period. After provision of written informed consent, subject eligibility for study entry will be assessed. Subjects who have been assessed to be eligible for the study, based on screening evaluations, will be admitted to the in-patient unit on Day -1, the day before E24377A administration, for study orientation and to be monitored for any signs and symptoms of illness. At baseline (Day 1), subjects who remain eligible and are willing to participate will be enrolled in the study and receive a single administration of E24377A.

Subjects may remain in the in-patient unit until they meet discharge criteria, see Section 9.4.8.2. Routine discharge is scheduled for Day 9, dependent upon 2 consecutive negative stool culture results. During the in-patient period, subjects will be examined daily for signs and symptoms of ETEC illness. Vital signs will be measured at least 3 times daily starting on Day 1, and postural blood pressure and pulse will be measured as needed. All stools will be collected and evaluated, weighed, and graded by the study staff. Up to 3 stools and/or rectal swabs collected each day will be cultured.

If the subject develops any symptom suggestive of diarrhea, they will be encouraged to drink liquids. If a subject vomits or is unable to consume an adequate volume of liquids, then intravenous (IV) fluids may be administered.

In order to reduce the risk of secondary infection after discharge, all subjects will receive a 3-day course of antibiotics. The subject will begin a 3-day course of ciprofloxacin 500 mg by mouth twice daily (or azithromycin 500 mg by mouth daily for three days, or amoxicillin 500 mg by mouth three times daily for three days if the subject was allergic to or unwilling to take

ciprofloxacin per the PI discretion) on the morning of Day 6, or sooner if the subject has met the criteria for early antibiotic treatment (see Section 9.4.8.2).

Subjects will be discharged from the in-patient unit if determined eligible for discharge (2 stools negative for the challenge organism by culture, having received at least 2 doses of antibiotics, with improvement or resolution of symptoms). Subjects who meet the criteria for early antibiotic treatment, may be discharged from the in-patient unit prior to Day 9.

After discharge from the in-patient unit, all subjects will attend the scheduled follow-up visit/contact on an out-patient basis on Day 29 (physical visit) and Day 180 (phone).

The analyses for the exploratory endpoints may continue after the study database has been locked.

The timing and frequency of study visits and assessments are presented in the schedule of events in Table 1.

Table 1. Schedule of Events (Subject Visit Schedule and assessments)

	Study Specific Screen	Inpatient period										Out-patient visit	Phone call
Study Day	Scr -60 to -2	-1	1	2	3	4	5	6	7	8	9	29	180
Informed Consent and Comprehension Test	X												
Demographics	X												
Inclusion/Exclusion Criteria	X	X	X										
Medical History	X	X											
Functional Bowel Survey	X												X
COVID-19 Test		X											
Full Physical Exam	X	X											
Focused Physical Exam			X	X	X	X	X	X	X	X	X	(X)[1]	
Vital Signs[2]	X	X	X	X	X	X	X	X	X	X	X	X	
Serology (HIV, HBsAg, and HCV)	X[3]												
CBC w/diff	X	X[4]											
Clinical Chemistry	X	X[4]											
ABO Type	X												
IgA deficiency screen	X[3]												
Serum HCG Pregnancy Test[5]	X	X											
Urine Drug Screen	X												
Urine Pregnancy Test[5]			(X)									X	
Enrollment			X										
E24377A challenge			X										
Prior and concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	
Interim Medical Interview[6]			X	X	X	X	X	X	X	X	X	X	X
AE			X	X	X	X	X	X	X	X	X	X	
SAE/AESI			X	X	X	X	X	X	X	X	X	X	X
Start Antibiotic therapy								X[7]					
Stool collection for weighing and grading[8]			X	X	X	X	X	X	X	X	(X)		
Stool culture[9]			X	X[10]	X	X[10]	X	X	X	X	(X)		
Fecal samples for exploratory immunogenicity endpoints		X	(X)[11]	X	X	X	X	X	X	X	(X)	X	
Blood Samples for exploratory Immunogenicity endpoints		X	(X)[11]		X	X				X		X	

Whole blood for ALS/Memory B Cells/PaxGene		X	(X)[11]			X				X		X	
ETEC Impact on ADLs								X					
Planned Discharge											X[12]		
Follow-Up Telephone Contact													X
Study Completion													X

Note: (X) denotes optional event or procedure
Note: Procedures to be performed at an unscheduled visit are described in section 9.5.6.6. Procedures to be performed in case of an early termination are described in section 9.5.6.8.
[1] Physical exam on Day 29 optional per PI discretion.
[2] Pulse, sitting, lying or standing blood pressure and temperature will be measured at least 3 times daily starting on Day 1. In addition, postural blood pressure and pulse will be measured if suspicion or evidence of hypovolemia (based on assessment by clinician). Vital signs may be measured more frequently if the subject is ill.
[3] Must be collected from Day -30 to Day -2.
[4] Hematology and clinical chemistry will be performed at Day -1 if not performed within the previous 7 days.
[5] Serum HCG pregnancy tests will be performed for people of child-bearing potential. Urine HCG will be done if serum results from admission day (Day -1) are not available.
[6] During the inpatient and outpatient phases of the study, the interview will be used to update baseline medical history, monitor safety, and to confirm ongoing eligibility.
[7] Subjects meeting the criteria for early antibiotic treatment per the discretion of an investigator may start this treatment prior to Day 6.
[8] Weighing and grading ends once participant has two consecutive ETEC negative cultures, unless there is a clinical indication to continue.
[9] Once a participant has two stools in a row negative for *ETEC,* their stools will no longer be cultured.
[10]Quantitative stool cultures (cfu per gram of stool) will be collected on Days 2 and 4.
[11]To be collected before challenge if not taken on Day -1.
[12] Anticipated discharge date. Participant may be discharged earlier or later depending on when they meet discharge criteria and per PI discretion.

9.1.1 Stopping rules/Discontinuation Criteria

Scandinavian Biopharma AB has the right to close the study at any time, although this should occur only after consulting involved parties. The IRB and the FDA must be informed. Events that may trigger premature termination of the study include, but are not limited to: safety reasons, results of any interim analysis, non-compliance with the protocol, change in development plans for the subsequent planned vaccination and challenge study, slow recruitment and poor quality data.

9.1.2 Internal Safety Review Committee (iSRC)

There will be no iSRC for this study.

9.2 Rationale for study design, doses and control group

For this pre-study and the planned CHIM study involving the ETVAX® vaccine, the E24377A ETEC strain is considered to be the best suited as (a) there is a fair amount of experience with E24377A (third most commonly used challenge strain [22], (b) the disease severity score is predominantly moderate [23] and (c) the time to diarrhea is with a median of 25 hours post-

challenge also intermediate compared to common ETEC challenge strains (e.g. 47 hours for H10407 and 13 hours for B7A) and, therefore well suited for a 5-10 day in-house challenge period [23].

The 0807 GMP lot of the ETEC E24377A strain has been used in previous CHIM studies to assess the efficacy of other candidate ETEC vaccines [18, 24]. The *E. coli* ETEC strain E24377A was first used in human challenge studies in the early 1980s [25]. Table 2 below presents a summary of the previous use of the selected challenge strain E24377A (*E. coli* O139: H28; LT^+ ST^+; CS1+ CS3) in CHIM studies. Challenge doses ranging from $6x10^8$ to $4x10^9$ were used with overall diarrheal attack rates around 80% and moderate to severe ETEC illness around 60-81%. The pre-study to precede the proposed CHIM study is planned to estimate the incidence of moderate and severe diarrhea among participants challenged with $4x10^9$ cfu of the *E. coli* E24377A strain.

No serious adverse events occurred in any of the studies listed in Table 2.

Table 2. Strain E24377A used in CHIM studies

Publication	Number Placebo subjects	Challenge Dose (cfu)	GMP status for challenge strain	Diarrhea Attack rate (%)	Comment
Levine 1984 [25]	14	$5x10^8$	Research grade	64%	-
Levine 1986 [26]	6	$5x10^8$	Research grade	100%	-
Tacket 1994 [27]	10	$3x10^9$	Research grade	100%	-
Tacket 1999 [28]	10	$1x10^8$	Research grade	30%	-
McKenzie 2007 [18]	20	$6x10^8$	Lot 0807 cGMP grade	100% [75%*]	LT patch vaccine trial
McKenzie 2008 [24]	10	$7x10^8$	Lot 0807 cGMP grade	80% [60%*]	Challenge dose-finding trial
	10	$4x10^9$		80% [70%*]	
	16	$3x10^9$	Lot 0807 cGMP grade	81% [81%*]	PTL-003 live-attenuated ETEC vaccine trial

* Attack rate of moderate to severe ETEC illness (moderate to severe diarrhea or mild diarrhea with moderate to severe GI symptoms) [18, 24]

E24377A was, in the early 1980s, isolated from a traveler returning from Egypt with an ETEC diarrheal illness [18], and it shares the CS3 and LT antigens with one of the *E. coli* strains the ETVAX® vaccine but is of a different O and H antigen type. E24377A harbors two colonization factors, CS1 and CS3, as well as the enterotoxins LT and STh. Since not only the colonization factors (CS1, CS3) and toxins (LT, ST) are expressed but also other common virulence factors (data on file from bioinformatic analysis performed [redacted] E24377A is considered a representative strain for conducting a CHIM study involving the ETVAX® vaccine.

The CS1+CS3 CF combination of virulence factors are well-represented in field isolates and contribute to virulence/infectivity/disease severity throughout the world. They are seen in ETEC strains causing disease in both adults and children globally [19]. A detailed analysis of the genes

encoding CS1, CS3, LT and ST in E24377A show that they are identical or highly similar (>99%) in comparison to the respective genes in other CS1+CS3 ETEC strains.

Provoked diarrheal illnesses after challenge in studies with the same preparation (lot 0807) of strain E24377A are presented in Table 3. The cGMP grade preparation of the ETEC strain E24377A (lot 0807) has been used in previous CHIM studies [18, 24], and triggered at doses of $6x10^8$ – $4x10^9$ moderate to severe ETEC illness of 60-81% (Table 2).

Table 3. Clinical experience in naïve subjects challenged with ETEC E24377A (Lot 0807 cGMP grade)

Type of study	**CHIM trial McKenzie 2007** [18]	**Challenge dose-finding McKenzie 2008** [24]		**CHIM trial McKenzie 2008** [24]
No Subjects[1] Challenge dose (cfu)	N=20 $6x10^8$	N=10 $7x10^8$	N=10[2] $4x10^9$	N=16 $3x10^9$
Attack rate (overall) Attack rate (moderate/severe ETEC illness)[3]	20 (100%) 15 (75%)	80% 6 (60%)	80% 7 (70%)	13 (81%) 13 (81%)
Time to illness (mean hours)	29 h	47 h	26 h	29.6 h (7 -84)
Stool volume (mean ml or g)	585 (0 – 1821)[4]	740	1400	1009 (0 – 5085)
Mean No loose stools	4.7 (0 – 12)[4]	5	8	6.9 (0 -25)
No subjects with a key symptom[5]	NA	NA	NA	8 (50%)
Early antibiotics given	12/15 (80%)	NA	NA	8 (50%)
IV fluid treatment needed	6/15 (40%)	NA	NA	NA

NA = not available in publications; cfu = colony forming units
[1] Data in this table is from only placebo recipients or unimmunized subjects in the trials cited
[2] 10 subjects in this group is a composite of 5 subjects given $4.9x10^9$cfu and 5 subjects given $3.1x10^9$ cfu of 24377A
[3] Moderate to severe ETEC illness includes mild diarrhea with moderate to severe GI symptoms
[4] During 0-48h after challenge
[5] Moderate or severe nausea, vomiting, anorexia, or abdominal pain/cramps

9.3 Selection of study population

9.3.1 Recruitment

The study site will recruit subjects from the greater Baltimore, Washington DC and surrounding regions.

IRB approved advertising will be conducted utilizing a wide range of print, electronic, audio, video and/or social media. Subjects responding to the advertisements by a phone call or through the website will be pre-screened for eligibility based on a standard pre-screening questionnaire administered by a CIR recruiter. Some elements of the inclusion/exclusion criteria will be

discussed with the subject at that time and a preliminary assessment will be made regarding the individual´s eligibility for study participation.

9.3.2 Compensation for participation

Compensation will occur as detailed below. Compensation will be provided only for completed study procedures designated for compensatory payment. If a participant is eligible to participate in the investigational protocol after screening, and completes all study visits, procedures and follows all the rules, they will receive a total of $3,860.

Volunteers that present to the unit as an alternate, but are not admitted, will receive $200 for screening and $200 for presenting to the unit (a total of $400). Volunteers who are admitted to the unit overnight, but are discharged prior to challenge, will receive $200 for screening (if not already received) and $350 for staying on the inpatient unit overnight.

If a participant is not eligible for discharge by Day 9 because of illness or not having 2 consecutive negative stool culture results they will receive $350 per additional inpatient day.

9.3.3 Screening according to protocol CIR200

Initial in-person screenings will be conducted under a separate, IRB-Approved general screening protocol ("Screening of adult volunteers for eligibility to participate in clinical studies evaluating investigational vaccines, antimicrobial agents, other disease prevention measures or the pathogenesis of infectious agents" CIR 200, JHSPH IRB 00010083). Subjects will be made aware that the screening process may take several visits to complete.

Using this screening protocol, a medical history/physical exam and a series of clinical laboratory tests may be completed to rule out occult illness and pregnancy. These laboratory tests may include, but are not limited to, complete blood count (CBC), serum chemistries, hepatitis B antigen, hepatitis C antibody, HIV-1 antibody, IgA levels, serum hCG (for people of childbearing potential), and urine toxicology (drug screening). (Confirmatory testing will be performed on participants who test positive for hepatitis C or HIV-1 antigens.) Participants who have ≤ 2 mild (grade 1) non-hematologic abnormalities may be included if the PI determines that their participation will not present undue risk to the participant. Participants with > 2 mild abnormalities will not be included in the study. Participants with clinical laboratory abnormalities of greater than mild severity will not participate in this clinical trial. The clinical toxicity grading scale that will be used as a guideline is based on the scale used by the Division of AIDS (DAIDS) for adverse events and the guidance from the US Food and Drug Administration (FDA) Center for Biologics Evaluation and Research. If any additional safety labs are performed, either scale may be utilized.

Participants who are eligible after completing the general screening assessment may be asked to complete study-specific screening under this protocol.

9.3.4 Number of subjects

Up to 30 (minimum 25) healthy subjects will be enrolled in the study. Since there is a maximum capacity of 30 subjects for the in-patient period, the number of subjects who receive the

E24377A strain will not exceed 30; however, additional alternate subjects may be recruited to fill in for subjects who are ineligible or chose to leave prior to challenge receipt.

9.3.5 Screening and enrollment log

The clinic will keep a log of all subjects screened and enrolled. The reason for screen failure should be stated for all subjects screened but not enrolled. The reason for withdrawal should be stated for all subjects enrolled but not completed.

Subjects who have signed the CIR200 ICF will be assigned a CIR200 screening number. Subjects who sign the study specific consent form will be assigned a study specific subject number.

9.3.6 Inclusion criteria

For inclusion in the study, subjects must fulfil all the following criteria:

1. Healthy adults between 18 and 50 years of age, inclusive, at the time of signing the informed consent.
2. General good health, without clinically significant medical history, physical examination findings or clinical laboratory abnormalities per clinical judgment of PI.
3. Negative pregnancy test at screening and prior to challenge for people of childbearing potential. People of childbearing potential must agree to use an efficacious hormonal or barrier method of birth control during the study. Abstinence is acceptable. People of childbearing potential unable to bear children must have this documented (e.g. tubal ligation or hysterectomy) or must have negative pregnancy tests at screening and prior to challenge.
4. Willingness to participate in the study after all aspects of the protocol have been explained and written informed consent obtained.
5. Completion of a training session and demonstrated comprehension of the protocol procedures and knowledge of ETEC associated illness by passing a written examination (70% pass score).
6. Availability for the study duration, including planned follow-up visit/contact.

9.3.7 Exclusion criteria

Subjects must not enter the study if any of the following exclusion criteria are fulfilled:

1. Presence of a significant medical or psychiatric condition which in the opinion of the investigator precludes participation in the study, including gastrointestinal disease (gastritis, irritable bowel disease as suggested by Rome III criteria or medical diagnosis, inflammatory bowel disease). Some medical conditions which are adequately treated and stable would not preclude entry into the study. These conditions might include stable asthma controlled with inhalers or mild hypertension stably controlled.
2. Significant abnormalities in screening haematology, or serum chemistry as determined by PI.
3. Presence in the serum of HIV antibody, HbsAg, or HCV antibody with confirmation of infection (e.g. by HCV PCR).
4. Evidence of IgA deficiency (serum IgA < 7 mg/dl or limit of detection of assay).

5. Evidence of current alcohol or drug dependence.
6. Subjects whose Body Mass Index (BMI) is less than 19.0 or greater than 37.0 (kg/m^2).
7. Recent vaccination or receipt of an investigational product (within 30 days before challenge) or intended vaccination or receipt of investigational products until 60 days after challenge, with the exception of licensed vaccine for influenza or SARS-CoV-2 vaccination that may be given up to 7 days prior to challenge.
8. Positive test for SARS-CoV-2 at arrival to the unit on the day of in-patient admission.
9. Intention to donate blood or blood products within one month following the completion of study participation (note: The Red Cross will not allow blood donations for 1 year following participation in an investigational research study).
10. Any other criteria which, in the investigator's opinion, would compromise the ability of the subject to participate in the study, the safety of the study, or the results of the study.
11. Abnormal stool pattern (fewer than 3 per week or more than 3 per day).
12. Regular (≥ weekly) use of laxatives, antacids, or other agents to lower stomach acidity.
13. Use of any medication known to affect the immune function (e.g., corticosteroids and others) within 30 days preceding the challenge or planned use during the active study period. Use of inhaled or topical steroids may be permitted per PI discretion.
14. History of microbiologically confirmed ETEC infection in the last 3 years.
15. Occupational handling of ETEC currently, or in the past 3 years.
16. Travel to countries where ETEC infection is endemic (most of the developing world) within two years prior to dosing OR visit for > two months in ETEC endemic countries during the last 10 years, OR planned travel to endemic countries prior to study day 180.
17. Vaccination for or ingestion of ETEC, cholera, or LT toxin within 5 years prior to dosing.
18. Use of antibiotics during the 14 days before challenge dosing or proton pump inhibitors, H2 blockers or antacids within 48 hours prior to challenge dosing.
19. History of diarrhea in the 7 days prior to challenge (outpatient diarrhea is defined as ≥ 3 unformed (grade 3 or greater) loose stools in 24 hours).
20. Known allergy to two of the three following antibiotics: ciprofloxacin, amoxicillin, and/or azithromycin.

9.3.8 Removal of subjects from therapy or assessment

Subjects are free to discontinue their participation in the study at any time and for whatever reason without affecting their right to an appropriate follow-up investigation or their future care.

Subjects may be discontinued from the study at any time at the discretion of the Investigator for any of the following reasons:

- Severe non-compliance to the CSP procedures, as judged by the Investigator and/or SBH
- Subject is lost to follow-up (at least 3 attempts will be made to contact them, then a certified letter will be sent to their last known address)
- Significant AEs posing a risk for the subject, as judged by the Investigator and/or SBH

The primary reason for withdrawal / discontinuation must be specified in the eCRF and medical records.

After administration of E24377A, the subject will be encouraged to remain in the in-patient unit for the protocol-specified period (i.e., until they meet discharge criteria of 2 stools negative for challenge; see Section 9.4.8.2) and return for the scheduled out-patient study site visit on Day 29, and be available for a final contact by phone approximately 6 months after challenge. Furthermore, subjects will be encouraged to take the full course of antibiotics in order to reduce the risk of secondary infection after discharge.

If a subject expresses interest in withdrawing from the study after E24377A administration but before completion of the in-patient period, then the subject will be started on antibiotic therapy and encouraged to remain in the in-patient unit until they are no longer infected, as determined by negative stool culture. If the participant is amendable to returning for any or all in-person outpatient visits, they will be considered "off treatment" but not terminated from the study.

However, if the subject elects to withdraw from the study immediately, then the subject will receive a single dose of ciprofloxacin 500 mg prior to discharge from the in-patient unit and be provided with the remaining 5 doses of ciprofloxacin 500 mg twice daily (BID). They will be encouraged to bring stools samples in to ensure clearance of the challenge organism.

If a subject terminates the study before Day 29, every effort will be made to have the subject undergo the procedures as described in Section 9.5.6.4 for the follow-up visit.

In case an early termination visit as described above is not possible, a follow-up safety phone call should be made as soon as possible after termination to capture any SAEs or AESIs since the last study visit, if possible.

9.4 Treatments

9.4.1 Identity of Test Product

The E24377A (lot 0807) challenge strain was manufactured and supplied by Walter Reed Army Institute of Research (WRAIR), Biological Production Facility, Silver Spring, MD, (MF 11636). Under current Good Manufacturing practice (cGMP) on August 31, 2000 and released on April 17, 2001 for use in a CHIM study. Vials of the WRAIR Production Cell Bank have been stored (at -80º C $\pm$10º C) at the WRAIR Pilot BioProduction Facility since their cGMP manufacture. Further testing of the challenge strain to re-confirm its characteristics has been performed to fulfill current quality and purity standards.

For the purposes of this study, all subjects will be challenged with an inoculum prepared by the CIR JHSPH Enteric Vaccine Research Lab under the supervision of the Investigational Drug Service (IDS) from freshly plated, grown organisms. This approach was used in all previously conducted clinical studies in which the E2477A challenge strain was used. Details of the challenge strain preparation are provided in the Study Specific Procedure CIR357 – SSP 001.

9.4.2 Packaging, labelling and storage of test product

Following receipt of the frozen challenge strain from WRAIR, it will be stored at the CIR JHSPH Enteric Vaccine Research Lab in accordance with GCP and GMP requirements and will be inaccessible to unauthorized personnel.

9.4.3 Administration of Challenge strain E23477A

Each subject will receive a single administration of approximately 4 x10^9 cfu of the ETEC strain E24377A (lot 0807) by mouth at baseline (Day 1).

A sodium bicarbonate buffer solution of 2 g/150 mL water will be prepared by research lab staff under the supervision of an IDS pharmacist. Each subject will drink 120 mL of this sodium bicarbonate buffer (in order to neutralize gastric acidity) one to two minutes prior to ingesting the challenge inoculum. Thereafter, subjects will drink the challenge inoculum (approximately 4 x10^9 cfu) dissolved in the remaining 30 mL of buffer.

All subjects will be fasted for approximately 90 minutes before challenge and for approximately 90 minutes after challenge.

9.4.4 Product accountability

The study site will maintain a Challenge Dispensing document in each participant chart detailing the dates and quantities of challenge administration to each subject. Records will be maintained that includes subject number, administration date and amount administered. This documentation will be available to the designated CRA to verify test product accountability during the study.

Any unused challenge strain will be accounted for and destroyed as per Sponsor and SSP instructions.

9.4.5 Method of assigning subjects to treatment groups

Not applicable

9.4.6 Blinding

Not applicable

9.4.7 Emergency decoding of blinded treatment

Not applicable

9.4.8 Prior and concomitant therapy

9.4.8.1 Prohibited medications

The use of the following therapies are prohibited and exclusionary for study enrollment, as specified below:

- Recent vaccination or receipt of an investigational product (within 30 days before challenge) or intended vaccination or receipt of investigational products until 60 days after challenge, with the exception of licensed vaccine for influenza or SARS-CoV-2 vaccination that may be given up to 7 days prior to challenge.
- Regular (≥ weekly) use of laxatives, antacids, or other agents to lower stomach acidity.

- Use of any medication known to affect the immune function (e.g. corticosteroids and others) within 30 days preceding the challenge or planned use during the active study period. Use of inhaled or topical steroids may be permitted per PI discretion.
- Vaccination for or ingestion of ETEC, cholera, or LT toxin within 5 years prior to dosing.
- Use of antibiotics during the 14 days before challenge dosing or proton pump inhibitors, H2 blockers or antacids within 48 hours prior to challenge dosing.

9.4.8.2 Concomitant therapy during the study

During the study, all subjects will receive a 3-day course of antibiotics.

On the morning of Day 6 (or sooner if the subject met the criteria for early antibiotic treatment; see below), all subjects will start a 3-day course of ciprofloxacin 500 mg BID.

Subjects with an allergy to or who are unwilling to take a fluoroquinolone, may receive azithromycin 500 mg by mouth daily for three days or amoxicillin 500 mg by mouth three times daily for three days.

If, because of illness, a subject is unable to take oral antibiotics, intravenous antibiotics may be given at an appropriate dose based on weight and clinical status.

Criteria for early antibiotic treatment

Subjects meeting any of the following criteria before Day 6 will start antibiotic treatment at that time:

- Severe diarrhea as defined as >800 grams of grade 3-5 stools within a rolling 24 hour-period (see Section 9.5.3.1.2).
- Stool output consistent with moderate diarrhea for 48 hours
- Diarrhea of any severity AND 2 or more of the following symptoms: severe abdominal pain, severe abdominal cramps, severe nausea, severe headache, severe myalgias, severe arthralgia), any fever (≥ 100.4°F, 38.0°C), or any vomiting
- Any fever ≥ 102.1°F (39.0°C)
- A study clinician determines early treatment is warranted for any other reason

Subjects who meet the criteria for early antibiotic treatment, may be discharged from the in-patient unit earlier than anticipated upon meeting discharge criteria. Discharge criteria include at least 2 stools negative for the challenge organism, having taken at least 2 doses of antibiotics, and have no or improved symptoms.

Subjects taking any other non-antibiotic medication on a chronic basis may continue with that medication unless directed otherwise by a study clinician. All such medication will be collected on admission to the inpatient facility and will be dispensed by study staff until discharge.

Rehydration

Subjects passing grade 3-5 stools post-challenge will be asked to drink liquids, at the same volume as their stool output.

A subject may be administered IV fluids if they:

- Experience abrupt onset of diarrhea, defined as passage of an initial loose/liquid stool of >300 g, or >400 g of loose/liquid stools over 2 hours in conjunction with other symptoms, as determined by PI or designee.
- Become hypovolemic, defined as confirmed supine systolic blood pressure (BP) < 90 mmHg and associated symptoms, or significant light-headedness on standing, with a confirmed postural change in BP or pulse.
- A decrease in systolic BP or diastolic BP of > 20 mmHg or increase in pulse of > 30 beats/minute takes place when measured lying down vs. two minutes after standing.
- If determined necessary by the study provider, e.g., diarrhea with nausea/vomiting and unable to drink enough to keep up with output, or other reason.

All concomitant medications administered including antibiotics and IV fluids will be documented in the subject´s medical record and in the eCRF.

Treatment for Vomiting

Treatment for severe nausea or vomiting may be needed. Participants who experience severe nausea or vomiting may be given oral or intravenous (IV) ondansetron (Zofran).

Treatment for Fever or Pain

Acetaminophen, ibuprofen, or naproxen may be used to treat fever or pain.

Other Treatments

In addition to above medications, other medications may be prescribed by the PI or designees, as needed for symptom control (e.g., heartburn), or if a medical problem arises (allergic reaction, insomnia). The most commonly utilized medication, including the indication are as follows:

Medication	**Dosage**	**Indication**	
Ondansetron oral dissolving tablet	4 mg, 8 mg once, can be repeated if not better in 30 minutes	Nausea/vomiting	
Ondansetron IV	0.15mg/kg IV x1	Vomiting	Max 16mg/dose
Acetaminophen	500 -1000 mg q 4-6 hours	Pain, fever	Max 4000 mg/day
Ibuprofen	400 – 800 mg q 6-8 hours	Pain, fever	Max 2400 mg/day
Naproxen sodium	220 – 440 mg q 12 hours	Pain, fever	Max 660 mg/day
Naproxen	250-500mg BID	Pain, fever	Max 1000 mg/day
Diphenhydramine	25-50mg oral q 6 hours PRN	Itching, insomnia	
Antacid/Antigas Liquid (Maalox, Mylanta or Generic)	As directed	Heartburn, indigestion	
Calcium Carbonate	As directed	Heartburn, indigestion	

9.4.9 Treatment compliance

The ingestion of sodium bicarbonate buffer solution and the challenge inoculum dissolved in sodium bicarbonate buffer will be supervised by personnel at the study site to ensure compliance.

9.5 Study assessments

The timing and frequency of study visits and assessments are presented in the schedule of events in Table 1.

Each study assessment/procedure is described in the sections below.

9.5.1 Demographics and other baseline characteristics

9.5.1.1 Demographic information

Demographic and baseline clinical data including date of birth, biological sex, gender, race, height, weight, body mass index (BMI), pulse rate, blood pressure (systolic/diastolic) and temperature will be recorded. In addition, information about previous SARS-CoV-2 vaccinations is collected from all participants.

9.5.1.2 Medical history

Medical history will be obtained in order to verify that the eligibility criteria are met. Medical records will not be requested unless there is a need to clarify a question in the participant's medical history or if the participant had an intercurrent illness or injury requiring medical care during the study. Informed consent for the medical release will be obtained from each participant during screening.

9.5.2 Safety assessments

9.5.2.1 Adverse Events

9.5.2.1.1 Definitions

Adverse Event (AE)
An AE is any untoward medical occurrence in a clinical study subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including clinically significant abnormal values from relevant tests, such as vital signs), symptom, or disease temporally associated with the use of an investigational product, regardless of whether it is considered related to the investigational product.
In this study the challenge strain is considered to be an investigational product and the causal relationship to the ingestion of the challenge strain will be assessed.

In addition, the causal relationship between an AE and treatment with antibiotics (not an investigational product) and/or study procedures will be assessed.

Clarifications:
Diagnostic and therapeutic non-invasive and invasive procedures, such as surgery, should not be reported as AEs. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an AE. For example, an acute appendicitis that begins

during the AE reporting period should be reported as "acute appendicitis" and the resulting appendectomy noticed under *Comments*.

Stable, pre-existing conditions and/or elective procedures are not AEs (including any not recognized or not reported prior to study entry) and will be recorded in the Medical History.

Serious Adverse Event (SAE)

An SAE is any AE that:

- results in death
- is life-threatening (this refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death had it been more severe)
- requires inpatient hospitalization or prolongation of existing hospitalization[1]
- results in persistent or significant disability/incapacity[2]
- is a congenital anomaly/birth defect[3]
- is medically important (this refers to an event that may not be immediately life-threatening or result in death or hospitalization, but may jeopardize the subject or may require intervention to prevent any of the SAEs defined above)

[1]The participant has spent significant time, usually involving at least an overnight stay, at the hospital or emergency room for treatment that would not have been appropriate in a primary care office or outpatient setting.
[2]There is a substantial disruption of the participant's ability to carry out normal life functions.
[3]Abortion, stillbirth and any malformation/disease must be reported as an SAE.

Examples of medically important events are intensive treatment in an emergency room for allergic bronchospasm or blood dyscrasias, convulsions that do not result in hospitalization, development of drug dependency and drug abuse.

Although not considered SAEs, cancers will be reported in the same way as SAEs.

Planned hospitalizations or surgical interventions for a condition that existed before the subject signed the ICF and that did not change in intensity are not SAEs.
If there is any doubt as to whether an AE meets the definition of an SAE, a conservative viewpoint must be taken, and the AE must be reported as an SAE.

Adverse Events of Special Interest (AESI)

An AE of special interest is an AE of scientific and medical concern specific to the Sponsor's product or program, for which ongoing monitoring and rapid communication by the investigator to the Sponsor can be appropriate. Such an event might warrant further investigation in order to characterize and understand it. The development of Irritable Bowel Syndrome or other continuing gastrointestinal condition will be considered an AESI.

Serious Adverse Reaction (SAR)

The term SAR is to be used whenever the Investigator assessed the SAE as possibly or probably related to the investigational product. In this study the challenge strain is considered to be an investigational product and the causal relationship to the ingestion of the challenge strain will be assessed.

Suspected Unexpected Serious Adverse Reaction (SUSAR)
A SUSAR is any SAR whose nature or intensity is not consistent with the current version of the Investigator's Brochure (IB).

9.5.2.1.2 Assessment of severity/intensity

AEs regarding vital signs/test results listed below will be rated according to the Table 4, Table 5 and Table 6.

Table 4. Reference Ranges and Adverse Event Rating for Vital Signs Parameters

Vital Signs	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	Potentially Life Threatening (Grade 4)
Tachycardia	101–115	116-130	>130	ER visit or hospitalization for arrhythmia
Bradycardia	50-54[a]	45–49	<45	ER visit or hospitalization for arrhythmia
Fever (°C) (°F)	38.0–38.4 100.4–101.1	38.5–38.9 101.2–102.0	39.0-40 102.1- 104	>40 >104
Hypertension (systolic, mm Hg)	141–150	151 – 155	>155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic, mm Hg)	91–95	96 – 100	>100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic, mm Hg)[b]	85–89	80 – 84	<80	ER visit or hospitalization for hypotensive shock

[a] Grade 1 bradycardia will not be considered an abnormality for this study unless judged to be clinically significant by the PI.
[b] If a participant has a baseline systolic BP in the 90's then a decrease in BP < 10 without associated clinical symptoms will not be considered an abnormality for this study unless judges to be clinically significant by the PI.

Table 5. Reference Ranges and Adverse Event Rating for Clinical Hematology Parameters

Test	**Normal**	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Hemoglobin (g/dL) (for screening purposes only)	M: LLN = 11.0 F: LLN = 10.5				
Hemoglobin, low		M: 10.0 to 10.9 F:9.5 to 10.4	M: 9.0 to <10.0 F: 8.5 to <9.5	M: 7.0 to <9.0 F: 6.5 to <8.5	M: <7.0 F:<6.5
Eosinophils (cells/mm^3)	15-500	551-1,500	1,501-5,000	> 5,000	Hospitalization or ER Visit
Leukocytes (white blood cells) (cells/mm^3)	2,500 to 10,800				
Leukopenia		2,000 to 2,499	1,500 to 1,999	1,000 to 1,499	< 1,000

Leukocytosis		10,801-15,000	15,001-20,000	20,001- 25,000	>25,000
Lymphocytes, low (cells/mm^3)	>650	600 to <650	500 to <600	350 to <500	<350
Neutrophils, low (cells/mm^3)	>1,000	800 to 1,000	600 to 799	400 to 599	<400
Platelets decreased (cells/mm^3)	≥125,000	100,000 to <124,999	50,000 to <100,000	25,000 to <50,000	<25,000

Table 6. Reference Ranges and Adverse Event Rating for Blood Chemistry Parameters

Test	**Normal**	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
BUN (elevation)	7-25	26-28	29-31	> 31	Requires dialysis
Creatinine (elevation)[a]	M: 0.7-1.4 F: 0.5-1.1	1.1-1.3 x ULN	> 1.3 to 1.8 x ULN OR Increase of > 0.3 mg/dL above baseline	> 1.8 to <3.5 x ULN OR Increase of 1.5 < 2.0 x above baseline	≥ 3.5 x ULN OR Increase of ≥ 2.0 x participant's baseline
Glucose, Random (mg/dL)	65 to115				
Hypoglycemia		55 to 64	40 to <55	30 to <40	<30
Hyperglycemia		116 to 160	>160 to 250	>250 to 500	>500
Potassium (mEq/L; *mmol/L*)	3.4 to 5.6				
Hypokalemia		3.0 to < 3.4	2.5 to <3.0	2.0 to <2.5	<2.0
Hyperkalemia		5.6 to <6.0	6.0 to <6.5	6.5 to <7.0	≥7.0
SGPT/ALT (elevation)	M:9 to 46 F: 6 to 29	1.25 to <2.5 x ULN	2.5 to <5.0 x ULN	5.0 to <10 x ULN	≥ 10x ULN
Sodium (mEq/L; *mmol/L*)	136 to145				
Hyponatremia		130 to <135	125 to <130	121 to <125	≤120
Hypernatremia		146 to <150	150 to <154	154 to <160	≥ 160

[a] Will be graded as the highest grade met by either criterion.

For other events including other ETEC disease-specific expected events where no pre-specified definition of severity is available, the Investigator will assess the severity of AEs and expected

events using his/her clinical expertise and judgement based on the most appropriate description below:

Mild	(Grade 1) The AE does not interfere in a significant manner with the subject's normal functioning level. It may be an annoyance.
Moderate	(Grade 2) The AE produces some impairment of function but not hazardous to health. It is uncomfortable and/or an embarrassment.
Severe	(Grade 3) The AE produces significant impairment of functioning or incapacitation and/or it is a hazard to the subject.
Potentially Life-threatening	(Grade 4) Potentially life-threatening event.

If the severity rating for an ongoing unsolicited AE changes before the event resolves, the highest severity will be recorded.

9.5.2.1.3 Assessment of causal relationship

The Investigator must assess the *causal relationship* between an AE and inoculation of the challenge strain using the definitions below and record it on the *Adverse Event Form* in the CRF as well as on the *Serious Adverse Event Report Form*, if applicable:

- Related – There is a reasonable possibility the test product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the test product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the test product caused the event.

In addition, the causal relationship between an AE and treatment with antibiotics (not an investigational product) and/or study procedures will be assessed.

9.5.2.1.4 Assessment of Outcome

The Investigator must assess the *outcome* of an AE using the definitions below and record it on the *Adverse Event Form* in the CRF:

- *Recovering/resolving* indicates that the event is improving.
- *Recovered/resolved with sequelae* the subject recuperated but retained pathological conditions resulting from the prior disease or injury.
- *Recovered/resolved* indicates that the event has improved or recuperated.
- *Not recovered/not resolved* indicates that the event has not improved or recuperated.
- *Fatal* the termination of life as a result of an AE.
- *Lost to Follow-up*

9.5.2.1.5 Collecting and recording of AEs

AEs identified using any of the following methods will be recorded:

- AEs spontaneously reported by the subject
- AEs observed by the Investigator or medical personnel
- AEs elicited based on non-leading questions from the Investigator or medical personnel

AE collection starts with the administration of the challenge strain and continues until 28 days after challenge (Day 29). At the phone call approximately 6 months after challenge, the AE collection will be limited to Adverse Events of Special Interest (AESIs), SAEs and new chronic illnesses (see Section 9.5.6.5). Any AE with start on Day 1 from the time of challenge, must be recorded with date with/or without start time.

All AEs, serious and non-serious, should be recorded in the CRFs.

9.5.2.1.6 Reporting of SAEs

Starting from administration of the challenge strain, all SAEs must be reported by the Investigator or designee in the Advantage eClinical System within 24 hours of knowledge of the event, regardless of the time that may have elapsed from the time the event occurred to when the Investigator first learns of it.

In the case of temporary lack of internet access or technical issue entering data into the Advantage eClinical System, a back-up system will be used for sending SAE notification via email to the CRO Medical Monitor/Safety Monitor using the email address [redacted] Please note that emails should not contain any participant PHI/PII. Any information relayed via email must be entered into the Advantage eClinical system as soon as possible after the system becomes available, no later than 3 days after the system becomes available.

The initial SAE report should contain as a minimum the following information:

- Subject identification1
- Test product
- Nature of the SAE and date of onset
- Name of the original reporter
- Preliminary assessment of causality

The initial report should then be followed by a detailed follow-up report as soon as possible but no later than three calendar days after the initial information was received.

Any unexpected SAEs associated with the use of the challenge strain (SAE assessed as possibly or probably related to the ingestion of the challenge strain), i.e. SUSAR, will be reported to the FDA and IRB according to applicable regulations (within 7 calendar days if fatal or life-threatening and within 15 calendar days if not fatal or life-threatening).

Further details are provided in a separate Safety Management Plan.

9.5.2.1.7 Follow-up of AEs/SAEs

In general, AEs and expected events must be followed up until resolution or stabilization. AEs will be recorded for 28 days after challenge, SAEs and AESIs for 6 months after challenge. At the follow-up visit (Day 29), information on new AEs/SAEs, if any, and stop dates for previously reported ongoing AEs must be recorded. At the follow-up contact approximately 6 months after challenge, information on new SAEs, if any, and stop dates for previously reported ongoing AEs must be recorded.

It is the responsibility of the Investigator to follow-up on all SAEs until the subject has recovered, stabilized, or recovered with sequelae, and to report to SBH all relevant new information using the same procedures and timelines as those for the initial report. Relevant information includes discharge summaries, autopsy reports, and medical consultation.
SAEs spontaneously reported by a subject to the Investigator within 30 days after the last follow-up assessment should be reported to SBH even after the clinical study has been finished, if, in the judgment of the Investigator, there might be an association between the event and the previous use of the test product or as a result of the investigation procedures.

9.5.2.1.8 Procedures in case of pregnancy

Subjects will be screened by pregnancy test before participation in this study and instructed to use effective contraception. Sexually active people of childbearing potential will have to use birth control (e.g. birth control pills, injection hormonal contraceptive, implant hormonal contraceptive, hormonal patch, IUD, sterilization by e.g. tubal ligation or hysterectomy, spermicidal products and barrier methods are considered acceptable). Abstinence from sexual intercourse that could result in pregnancy is acceptable.
Although not AEs, each pregnancy must be reported within 72 hours of site awareness to the sponsor and IRB. Pregnancies will be captured through Day 29. Pregnancy will be followed for outcome, and outcome will be reported (e.g., any premature terminations, elective or therapeutic, and any spontaneous abortions or stillbirths, as well as the health status of the mother and child including date of delivery and infant's gender, length and weight). In general, pregnancies should be followed until birth or outcome is known unless the participant withdraws their study consent or is otherwise lost to follow up.

9.5.2.1.9 Coding of AEs

All AEs will be coded according to the current Medical Dictionary for Regulatory Activities (MedDRA), and WHODrug dictionaries respectivelyby [redacted]

9.5.2.2 Clinical Laboratory assessments

Toxicology Screen

Urine samples for a toxicology screen will be collected during screening. Such samples will be tested for the presence of amphetamines, barbiturates, benzodiazepines, cocaine and metabolites, methadone, opiates, oxycodone and phencyclidine.

Serology

A blood sample for serology, including HIV antibody, HbsAg, and HCV antibody (with PCR confirmation for active disease) will be collected during screening.

Pregnancy Test

A blood sample for a serum β-HCG pregnancy test will be collected during screening and on admission from people of childbearing potential. Urine HCG will be done if serum results from

admission day (Day -1) are not available by the time of Challenge and at the Day 29 outpatient visit.

ABO Type and Rh Status

An ABO type and Rh status will be collected during screening.

Hematology and Clinical Chemistry

Blood samples will be collected for clinical laboratory evaluations during screening. Blood samples for clinical laboratory evaluations, as listed below, will also be collected at Day -1 if they were not performed within the previous 7 days.

Hematology (which will include):

Hemoglobin	White blood cell (WBC) count with differential
Hematocrit	Platelet count

Clinical Chemistry (which will include):

Blood urea nitrogen (BUN)	Potassium
Creatinine	Alanine transaminase (ALT)
Glucose	Sodium
Carbon dioxide	Chloride

Analysis of all screening blood and urine samples will be performed by Quest Diagnostics.

Samples & Shipping

The maximum volume of blood to be drawn will not exceed 550 mL over any 8-week period, which healthy adults should tolerate and which should not compromise the health of participants.

Blood and stool will be collected by trained personnel as per the Schedule of Procedures (Table 1) and per the MOP. They will be labelled with the participant study numbers, processed and stored on the inpatient unit or the clinic, and then packed and sent to the CIR laboratory at an agreed upon shipment schedule. All research bloodwork and stool samples, as well as the preparation of the live inoculum, will be carried out in the laboratory of the CIR in the JHSPH. Immunologic and other research assays (as per study schedule) will be carried out at the JHSPH CIR Enterics lab.

Samples will be stored and distributed in compliance with DOT/IATA standards, guidance from the JHU Biosafety Review, and based on the MOP. Samples collected under this protocol will be used to conduct safety, microbiologic, and immunogenicity evaluations and other research labs.

Urine b-HCG will be performed by the CIR under a CLIA waiver.

Additional results on blood work and the urine drug screen may be received due to the use of standard panels. Extraneous results will not be considered in determining a subject's eligibility,

although the PI or designee will contact subjects about any abnormal values deemed to be clinically significant.

Biospecimen Management
Any unused blood or stool specimens will be stored once the study is complete. Samples and data collected under this protocol may be used only for research purposes. Participant DNA present in samples will not be used for genetic testing, however genetic testing may be done on bacterial DNA. These samples will not be sold or used to make commercial products.

Access to research samples will be limited using locked freezers. Samples and data will be stored using codes assigned by the investigators or their designee(s). Data will be kept on password-protected computers. Only investigators or their designee(s) will have access to the samples and data.

Any other research or experimental treatments will be conducted under other protocols for which separate IRB review and approval will be obtained. At the completion of the protocol (termination), samples that remain at JHSPH and data may be transferred to a repository [redacted]

In the future, other investigators who may wish to study these samples and/or data. In that case, IRB approval will be sought prior to any sample sharing. Similarly, IRB approval will be obtained prior to sharing any clinical information, with or without patient identifiers that may be linked to samples being shared. The research use of stored, unlinked, or de-identified samples (for example, as a standard for immunological analyses) may be exempt from the need for prospective IRB review and approval. Exemption requests will be submitted in writing to the JHU IRB office, which is authorized to determine whether a research activity is exempt.

Any loss or unintentional destruction of samples, or data (for example, due to freezer malfunction, or missing case report forms [CRFs]) that compromises the scientific integrity of the study or poses a potential breach of confidentiality for the participant will be reported to the IRB.

Any future research use of these biological samples will require IRB approval. Subjects will be asked to consent for the future use of their specimens as part of consenting to participate in this study. Samples will be stored only with the participant's permission, which is a requirement for participation in the study. The participant may withdraw permission for future use of specimens at any time. If a participant withdraws his or her permission for future use of specimens, those specimens will be destroyed. In this case, the PI will ensure the destruction of all known remaining samples and will report this to both the participant and IRB. This decision may not affect the participant's participation in future protocols.

9.5.2.3 Physical examination

A complete physical examination [head, eyes, ears, nose, and throat (HEENT), heart, lungs, abdomen, skin, lymph nodes, neurological, and musculoskeletal systems] will be performed during screening and on admission.

During in-patient period, a focused physical exam will be conducted daily. Subjects will be examined by the investigator daily for signs and symptoms of ETEC illness. Examinations will be performed more frequently if the subject is ill.

Physical examination findings will only be collected in the eCRF when there are abnormal findings. Abnormal findings assessed prior to challenge will be documented as pre-existing medical conditions (Medical history form). Abnormal findings from the time of challenge onward will be documented as adverse events or expected events.

9.5.2.4 Vital signs

Vitals signs, including systolic and diastolic blood pressure (mmHg), pulse (beats/minute) and temperature (°C), will be measured at least 3 times daily during in-patient period. In addition, postural (supine to standing) blood pressure and pulse will be measured if indicated by clinical signs and symptoms. If tolerated, postural vital signs will be measured after the subject has been supine for 2 minutes, then again after 2 minutes of standing. Vital signs may be measured more frequently if the subject is ill or per the orders of the PI or designee.

Only key vital signs (measured on Day -1 and on Day 1 after challenge) will be collected in the eCRF. Additional vital signs may be collected in the eCRF per the investigator´s clinical decision; clinical findings may be reportable as AEs per provider discretion.

The following vital sign measurements are concerning for hypovolemia and will be closely monitored:

- Supine systolic blood pressure <90 mmHg.
- Postural decrease in systolic BP of >20 mmHg.
- Postural decrease in diastolic BP of >20 mmHg.
- Postural increase in pulse >30 beats/min.

9.5.3 Clinical and Microbiology assessments during in-patient period

9.5.3.1 Stool collection and Evaluation

During the in-patient period, all stools will be collected, weighed and graded in order to determine the occurrence and intensity of diarrhea.

9.5.3.1.1 Stool grading System

Stools will be graded according to the following criteria:

Grade 1 = firm, formed
Grade 2 = soft but still formed
Grade 3 = thick liquid
Grade 4 = thin liquid
Grade 5 = clear or translucent, watery

The end of a diarrheal episode occurs when a subject does not pass any grade 3-5 stool within 24 hours.

9.5.3.1.2 Diarrhea definitions

Diarrhea will be rated according to the following criteria:

Mild	2-3 grade 3-5 stools in 24 hours and ≤400 grams of grade 3-5 stools passed in a 24-hour period
Moderate	4-5 grade 3-5 stools in 24 hours or >400-800 grams of grade 3-5 stools passed in a 24-hour period
Severe	≥6 grade 3-5 stools in 24 hours or >800 grams of grade 3-5 stools passed in a 24-hour period

Up to 3 stools and/or rectal swabs each day will be cultured. Fecal cultures will be performed by the Enterics lab at JHSPH. Stools or swabs for culture will be taken to the JHSPH laboratory, where they will be inoculated directly onto MacConkey agar and onto MacConkey agar that had incorporated streptomycin selective for the inoculum strain. Up to 10 colonies will be agglutinated using specific antisera. The level of fecal shedding (cfu per gram of stool) will be assessed by quantitative stool cultures on Days 2 and 4 post-challenge.

9.5.3.1.3 ETEC Disease Severity

A three-component disease score utilizing objective signs, subjective, symptoms and stool output will be analyzed for the entirety of the clinical illness (Table 7). The scoring goes from 0 (no diarrhea and no other signs or symptoms) to the maximum of 8 (severe ETEC disease) [31] .

Table 7. Disease Severity Score Components

Parameter	**Outcome**	**Score**
Objective signs	>1 episode of vomiting/24 hours OR any fever	2
	1 episode of vomiting AND no fever	1
	No vomiting AND no fever	0
Subjective symptoms	Moderate-severe lightheadedness OR Severe nausea, malaise, headache or abdominal cramps	2
	Mild lightheadedness OR Mild-moderate nausea, malaise, headache or abdominal cramps	1
	No 'subjective symptoms'	0
Diarrhea score	>1000 grams of grade 3-5 stool OR >12 grade 3-5 stools in 24 hours	4
	>600 to ≤1000 grams of grade 3-5 stool OR >7 to 12 grade 3-5 stools in 24 hours	3
	>400 to ≤600 grams of grade 3-5 stool OR >4 to ≤7 grade 3-5 stools in 24 hours	2
	>0 to ≤400 grams of grade 3-5 stool OR 1 to 4 grade 3-5 stools in 24 hours	1
	No grade 3-5 stools	0
1 gram of grade 3-5 stool is considered equivalent to 1 mL of grade 3-5 stool.		

9.5.3.1.4 ETEC Disease-specific solicited events

ETEC disease-specific solicited events will start being collected after challenge and will be assessed during inpatient physical examinations. Between Challenge and discharge, these pre-defined solicited events will be reported as adverse events and their relationship to the challenge strain, study procedures and/or antibiotics will be determined by the PI or designee.

ETEC Disease-specific subjective solicited events include the following symptoms:

- Malaise
- Loss of appetite
- Headache
- Chills
- Nausea
- Abdominal pain
- Abdominal cramps
- Myalgia
- Arthralgia
- Urgency of defecation
- Lightheadedness

The following solicited events will be documented via objective clinical assessments during the inpatient challenge phase:

1. Diarrhea (via stool logs)
2. Hypovolemia
3. Fever (oral temperature ≥ 100.4° F)
4. Vomiting

Table 8. Severity rating of ETEC Disease-specific expected events

Event	**Severity**[a]	**Parameter**
Diarrhea, based on highest output of loose[b] stools in any 24-hour period. (A diarrhea episode ends when there is a 24-hour window with no grade 3-5[b] stools.)	1	Mild: 2-3 loose[b] stools in 24 hours and ≤400 grams of loose[b] stools passed in a 24-hour period
	2	Moderate: 4 to 5 loose[b] stools in 24 hours or >400-800 grams of loose[b] stools passed in a 24-hour period
	3	Severe: 6 or more loose[b] stools in 24 hours or >800 grams of loose[b] stools passed in a 24-hour period
	4	Potentially life-threatening
Fever[c]	1	100.4ºF–101.1ºF (38.0–38.4ºC)
	2	101.2ºF–102.0ºF (38.5–38.9ºC)
	3	102.1ºF–104ºF (39.0–40.0ºC)
	4	>104ºF (>40.0ºC)
Vomiting	1	One episode within a 24-hour period
	2	Two episodes within a 24-hour period
	3	More than two episodes within a 24-hour period
	4	Potentially life-threatening consequence of emesis

[a]1=mild; 2=moderate; 3=severe 4=potentially life-threatening.
[b]Grades 3-5 stools are considered loose
[c]Oral temperature; no recent hot or cold beverages, eating or physical activity. If temperature is ≥ 100.4 °F every attempt should be made to
repeat within 20 minutes. If the repeat temperature is WNL and the investigator feels the repeated temperature is a more accurate reflection of the participant's real temperature a fever will not be entered into the eCRF.

If an ETEC Disease-specific expected event changes in severity, it should be reported as a new event with the new severity but with the same event description. For definitions regarding severity rating and assessment of causal relationship see Sections 9.5.2.1.2, 9.5.2.1.3 and Table 8.

For other events including other ETEC disease-specific expected events where no pre-specified definition of severity is available, the Investigator will assess the severity of events using his/her clinical expertise and judgement based on the most appropriate description stated in Section 9.5.2.1.2.

9.5.3.1.5 Subject´s assessment of impact of ETEC illness on daily activity

At Day 6 the subject will answer the following questions:

1. If traveling for vacation or business, would you have changed your itinerary?
2. Would you have stayed in bed (if so, how long)?

9.5.4 Additional assessments

9.5.4.1 Prior and concomitant medications

Medications taken by the subjects within 28 days prior to study screening until the follow-up visit (Day 29) will be documented in the subject´s medical record and recorded in the eCRF. Prescription medications, over-the-counter (OTC) medications, and herbal products should be asked for.

Prior and concomitant medication will be documented at the Screening visit. Changes in prior/concomitant medication compared to Screening will be documented throughout the study. For detailed information on prohibited and concomitant medication to be administered during the study, refer to Section 9.4.8.

Medication that was stopped before baseline (Day 1) will be classified as ‘prior medication’. Medications used from Day 1 will be classified as ‘concomitant medication’.

9.5.5 Exploratory assessments

- Immunogenicity of the challenge with enterotoxigenic *E. coli* (ETEC) strain E24377A will be evaluated as shown below:
  - Serum IgA and IgG antibody response to LT, CS1 and CS3 will be assessed.
  - ALS IgA antibody response to LT, CS1 and CS3 will be assessed.
  - Fecal IgA antibody response to LT, CS1 and CS3 will be assessed.
    IgA and IgG antibody levels will be determined by ELISA.
  - Systemic and intestinal inflammatory biomarkers will be evaluated.

- Fecal shedding (colony forming units per gram of stool) of the E24377A challenge strain will be evaluated post-challenge.

9.5.6 Schedule of Procedures

9.5.6.1 Screening

9.5.6.1.1 Screening according to general screening protocol

Subjects may be consented to a general screening protocol as per Section 9.3.3.

9.5.6.1.2 Study specific Screening (Day -60 to -2)

Healthy, potentially eligible and willing participants will be invited back to complete study specific screening.

The following assessments/procedures will be carried out:

- The participant will be provided information about the study, pass a comprehension test (see section 5.3) and provide signed informed consent before any study specific procedures are initiated
- Obtain demographic data including gender, race, height, weight, body mass index (BMI), date of birth and previous SARS-CoV-2 vaccination(s)
- Review medical history collected during CIR200 screening
- Review history of all current medications as well as those taken within the past 28 days
- Obtain responses to Functional Bowel Disorder Survey. The Functional Bowel Survey is an adaptation of Rome III guidelines utilized by the CIR to screen for gastrointestinal dysfunction per the CIR guideline.
- Obtain additional IRB-approved forms, which may include, but are not limited to the Alternate Agreement, Inpatient Unit Guidelines, Correct Hand Washing Procedure, HIPAA Medical Record Release Form, HIV Test Counseling and/or COVID Vaccination Form
- Perform complete physical examination
- Vital signs (temperature, pulse and blood pressure) will be measured
- Review inclusion/exclusion criteria to the extent possible
- Collect blood and urine samples (see Section 9.5.2.2)
- Obtain pregnancy test (serum β-HCG) from people of childbearing potential

9.5.6.2 In-patient period

9.5.6.2.1 Admission (Day -1)

- Inclusion/exclusion criteria (including test for SARS-CoV-2) will be reviewed to re-assess ongoing eligibility
- Perform a complete physical examination

- Collect blood samples if the clinical chemistry and hematology labs were not performed within the previous 7 days (see Section 9.5.2.2)
- Obtain pregnancy test (serum β-HCG) from people of childbearing potential
- Concomitant medications will be reviewed
- Vital signs (temperature, pulse and blood pressure) will be measured
- Medical history will be reviewed
- Blood and fecal samples will be collected for immunogenicity endpoints (exploratory)

9.5.6.2.2 Challenge day (Day 1)

On the day of challenge, subjects will consume a light breakfast and then initiate a 90-minute fasting period.

The following assessments/procedures will be carried out before challenge:

- Eligibility criteria will be re-confirmed
- A focused physical examination and evaluation will be performed to ensure that there are no changes from admission and no exclusionary conditions have arisen
- Urine β-HCG pregnancy test for people of childbearing potential if serum results from admission day (Day -1) are not available
- Blood and fecal samples will be collected for immunogenicity endpoints (exploratory) before challenge if not taken on Day -1.
- Concomitant medications will be reviewed
- Approximately 1 minute prior to challenge, subjects will drink 120 mL sodium bicarbonate buffer
- For the challenge, subjects will drink 30 mL of the sodium bicarbonate buffer containing approximately 4 x10^9 cfu of the ETEC strain E24377A.
- Subjects will not be redosed if they have emesis after challenge, however this will be documented.
- Subjects will continue fasting for an additional 90 minutes post challenge
- Vital signs (temperature, pulse, blood pressure) be recorded 30 minutes after challenge.
- All stools will be collected for weighing and grading after challenge. Should there be grade 3-5 stools after challenge, they will be saved for culture.
- Record any AEs, AESIs and SAEs

9.5.6.2.3 Post Challenge phase (Day 2 to 8)

- Daily medical interview (including assessment of ETEC disease-specific solicited events) and focused physical examination
- Vital signs (temperature, pulse and blood pressure) will be measured at least 3 times daily

- All stools will be collected for weighing and grading. Up to 3 stool samples will be sent daily for culture. If a volunteer is unable to provide samples, they will be asked to provide a self-collected rectal swab (up to 3 per day). (If the participant does not produce a stool or enough stool on any day to obtain all samples, this will not be considered a protocol deviation.)

- Record any AEs, AESIs and SAEs
- Changes in concomitant medications will be captured
- Antibiotic treatment will be started on Day 6 (5 days after challenge) or according to criteria for early antibiotic treatment (see Section 9.4.8.2)
- Collect blood samples on Days 3, 4 and 8 for immunogenicity endpoints (exploratory)
- Collect daily fecal samples for immunogenicity endpoints (exploratory)
- Collect ETEC Impact on ADLs survey responses on Day 6

9.5.6.3 Discharge (Day 9)

Routine discharge is planned for study day 9. Procedures that must be completed prior to discharge include:

- Vital signs (temperature, pulse and blood pressure) will be measured (at least 1 set)
- Medical interview (including assessment of ETEC disease-specific solicited events) and focused physical examination
- Record any AE, AESIs and SAEs
- Changes in concomitant medications will be captured
- All stools will be collected for weighing and grading until participant is eligible for discharge. Collections will end once discharge criteria is met.

Some participants may be discharged prior to Day 9. Their discharge will also follow these procedures, with the addition of being dispensed any remaining doses of antibiotics and directions for their use. Early discharge participants will also be given directions for outpatient follow-up visits on Days 7 and 8 if applicable, see section 9.5.6.7.

Volunteers who do not meet discharge criteria by day 9 will remain on the unit and will continue to undergo the procedures listed in section 9.5.6.3.

9.5.6.4 Out-patient follow-up Visit (Day 29 ± 4 days)

Procedures this day will include:

- Vital signs (temperature, pulse and blood pressure) will be measured
- Urine β-HCG pregnancy test for people of childbearing potential
- Medical interview and review of concomitant medications and AEs/AESIs/SAEs
- Focused Physical exam if participants have complaints/AEs or symptoms

- Collect blood and fecal samples for immunogenicity endpoints (exploratory)

9.5.6.5 Follow-up contact (Day 180 ± 14 days)

This medical interview/contact will occur by phone and will include:

- Obtain responses to the Functional bowel disorder survey
- Participants will be questioned about their health status, including any new chronic illnesses, AESIs or SAEs

9.5.6.6 Unscheduled Visit

If an unscheduled visit occurs, a member of the clinical study team will interview and evaluate the subject to determine the cause of the visit and provide care as needed and information documented in the source data. Adverse events and concomitant medications will be reviewed. Clinical laboratory tests and physical examination may be done as indicated.

9.5.6.7 Early Discharge Outpatient Visits (Day 7 & 8)

If the participant meets the discharge criteria early and are determined to be eligible by an investigator, they will be considered discharged. Participants will be offered the options of remaining on the unit as a boarder or leaving the unit and returning on Day 7 and 8 (as applicable) to complete the outpatient procedures per the Schedule of Events (Table *1*). Boarders who remain on the unit will undergo the procedures required for outpatient visits. Boarders will continue to have their concomitant medications and antibiotics administered by staff and recorded on the CRF. Early discharged participants will be given stool collections kits so that they may bring fecal samples to outpatient visits. Fecal samples will be collected, processed and sent per Table 1. Stool cultures will not be sent.

If the participant does not meet the discharge criteria but elects to withdraw early from the inpatient period and is amendable to returning for any or all future study visits, they will be considered “off treatment” but not terminated from study

9.5.6.8 Early Termination Visit

If a subject terminates the study before Day 29, every effort will be made to have the subject undergo the procedures as described in Section 9.5.6.4 for the follow-up visit.

In case an early termination visit as described above is not possible, a follow-up safety phone call should be made as soon as possible after termination to capture any SAEs or AESIs since the last study visit, if possible.

The reason for premature termination must be documented in the subject´s medical records and in the eCRF.

9.5.7 Appropriateness of measurements

Standard safety assessment methods are used in this study and are very similar to procedures performed in all previously conducted clinical studies in which the E2477A challenge strain was used.

The purpose of the current study is to estimate the incidence of moderate and severe diarrhea among participants challenged with $4x10^9$ cfu of the *E. coli* E24377A strain. This dose is planned to be used in a subsequent randomized, double-blind, placebo-controlled phase 3 vaccination and CHIM study. In order to meet this objective, all stools will be collected during in-patient period from immediately after E24377A administration on Day 1 through meeting discharge criteria and examined, weighed, and graded by the study staff using standard criteria.

9.6 Data quality assurance

This study will be conducted in compliance with the CSP, relevant Standard Operating Procedures (SOPs), Good Clinical Practice (GCP) and the applicable regulatory requirement(s).

The Principal Investigator will provide SBH with all data produced during the study from the scheduled study assessments. He/she ensures the accuracy, completeness, legibility, and timelines of the data reported to SBH in the eCRF and in all required reports.

9.6.1 Monitoring

The study site will be [redacted] overseeing monitoring activities at times agreed on by the Investigator. It is the function of the Monitor to ascertain that all aspects of the CSP are complied with and that the conduct of the study conforms to applicable regulatory requirements and established rules for GCP.
At the time of each monitoring visit, the Monitor will review the eCRFs to ascertain that all items have been completed and that the data provided are accurate and obtained in the manner specified in the CSP.

The Monitor will also check that the data in the eCRF are consistent with the clinical records (Source Data Verification) and that study results are recorded completely and correctly.

The Monitor will check on the reporting of SAEs, procedures for test product accountability and record keeping. For this purpose, the Monitor must be given direct access to clinical records, original laboratory data, etc., as far as these relate to the study and without jeopardizing subject integrity. eCRFs for all included subjects must be made available to the Monitor for review.

Further details are provided in a separate Monitoring Plan.

9.6.2 Audits and inspections

Authorized representatives of SBH or a regulatory authority, may perform audits or inspections at the research clinic, including Source Data Verification (SDV). The purpose of an audit or inspection is to systematically and independently examine all study-related activities and documents, to determine whether these activities were conducted, and data were recorded, analyzed, and accurately reported according to the protocol, ICH-GCP guidelines and any applicable regulatory requirements. The Investigator will contact SBH immediately if contacted by a Regulatory Authority about an inspection at the site.

9.6.3 eCase Report Forms (eCRFs)

An eCRF must be completed and signed by authorized personnel for each included subject, according to the eCRF completion instructions.

All data must be entered in English. The eCRFs should always reflect the latest observations made during the subjects´ participation in the study. Therefore, the eCRFs should be completed as soon as possible during or after the subject's visit. Data generated during the inpatient period will be entered in a timely fashion after discharge of the volunteers.

The Investigator must verify that all data entries in the eCRFs are accurate and correct by signing the completed eCRF.

The completed eCRFs should be made available for checking of completeness and accuracy by the Monitor.

Please see Section 9.8 for further details.

9.6.4 Source Data

A separate source data document will be generated by the site before start of subject screening. The source documents will include all information appearing in the eCRFs.

The Investigator should guarantee access to source documents to the Monitor and Regulatory Authorities, if required.

9.6.5 Training of study staff

Before screening of the first subject the Monitor and/or Project Manager will perform an initiation visit at the study site. The requirements of the Clinical Study Protocol and related documents will be reviewed and discussed, and the study staff will be trained in any study specific procedures and system(s) utilized.

It is the responsibility of the Principal Investigator to ensure that all personnel involved in the study are fully informed of all relevant aspects of the study, and have a detailed knowledge of and training in the procedures that are to be executed by them, including good documentation practice. Any new information of relevance to the performance of this study must be forwarded to the staff involved in a timely manner.

The Investigator will keep a list of all personnel involved in the study together with their function and study related duties delegated. A Curriculum Vitae (CV) will be available for all CIR staff delegated study-specific duties.

9.7 Statistical methods and determination of sample size

A detailed statistical analysis plan (SAP) will be written and finalized in advance of database lock. The plan will follow the outline of the statistical analyses presented below, but details necessary to complete the statistical analyses will be given.

9.7.1 Demographics and baseline characteristics

9.7.1.1 Demographics

Descriptive statistics of demographics and other baseline characteristics will be presented.

9.7.1.2 Medical history and concomitant medication

Descriptive statistics of medical history, prior/concomitant medications, and on-study antibiotic treatment will be presented.

9.7.2 Analysis of Clinical and Microbiology endpoints

For the primary endpoint, the proportion of subjects with moderate and severe diarrhea (defined as ≥ 4 grade 3-5 stools **or** > 400 grams of grade 3-5 stools passed in a rolling 24-hour period), deemed attributable to ETEC by an adjudication committee, will be presented with point estimate and the corresponding two-sided 95% confidence interval. For secondary clinical and microbiology endpoints, descriptive statistics will be presented, including two-sided 95% confidence intervals where applicable. For time to event analysis (time to onset of diarrhea and time to primary endpoint) Kaplan-Meier time to event methods will be used.

9.7.2.1 Analysis of requirement for early antibiotic treatment

The number and percentage of subjects requiring early antibiotic treatment, defined as treatment beginning prior to Day 6, will be presented.

9.7.3 Analysis of safety

9.7.3.1 Adverse Events

AEs and SAEs will be recorded from administration of the challenge strain until the follow-up visit on Day 29. At the follow up contact (phone call) approximately 6 months after challenge, new SAEs, new chronic illnesses (capture new diagnoses e.g. diabetes, hypertension, cancer) and AESIs (see Section 9.5.2.1.1) will be queried, and any open AEs and/or expected events will be followed up.

AEs will be coded using the current Medical Dictionary for Regulatory Activities (MedDRA) and summarized by Preferred Term (PT) and System Organ Class (SOC). The number of subjects reporting AEs, and the number of AEs reported will be presented. The events will be tabulated by SOC, PT and by severity and relationship to test product. AEs leading to study withdrawal and SAEs will also be presented in separate tabulations.

9.7.3.2 Vital signs and Physical examination findings

Key vital signs (those measured on Day -1 and 30 minutes after challenge) will be summarized.

9.7.4 Analysis of exploratory assessment

The exploratory assessments will be reported separately from the Clinical Study Report (CSR).

9.7.5 Analysis data sets

9.7.5.1 Full Analysis Set (FAS)

Unless otherwise stated, all statistical evaluations will be based on all subjects who have been challenged with E24377A (regardless if the whole intended challenge dose was ingested or a subject vomited just after ingestion) and have available data.

9.7.5.2 Determination of sample size

In preparation for a planned phase 3 vaccination and CHIM study, it is assumed based on previous clinical experience, see Table 2 and Table 3 [18, 24] that a pre-study in up to 30 subjects should be sufficient to estimate the incidence of moderate and severe diarrhea among participants challenged with $4x10^9$ cfu of the *E. coli* E24377A strain. With 25 subjects and an attack rate of 60% or 70%, the 95% confidence interval for the estimated incidence would be (0.39, 0,79) or (0.45, 0.87), respectively.

Since there is a maximum capacity of 30 subjects for the in-patient period, the number of subjects will not exceed 30.

9.7.6 Statistical/analytical issues

9.7.6.1 Adjustments for covariates

No adjustment for covariates in statistical analyses are planned.

9.7.6.2 Handling of dropouts or missing data

The handling of dropout and missing data will be provided in the SAP.

9.7.6.3 Multi-center studies

The study will be conducted at one site.

9.7.6.4 Multiple comparison/multiplicity

No formal statistical testing is planned for this study.

9.7.6.5 Active-control studies intended to show equivalence

Not applicable

9.7.6.6 Examination of subgroups

No examination of subgroups is planned

9.7.6.7 Interim analyses and data monitoring

An adjudication of challenge outcome data will be performed after completion of the in-patient period. Following a partial lock of the challenge outcome data, topline results will be generated before the last subject´s follow-up contact.

9.8 Outcome Adjudication Committee

An outcome adjudication committee will be used to judge if the diarrhea is attributable to ETEC and determine if a subject meets the primary endpoint by considering individual data as described in the adjudication charter.

The committee will be comprised of at least 3 individuals, who are experts on diarrheal illness case identification and pathogen diagnosis. The committee will also include a statistician/data analyst who will participate in the adjudication meeting but will not have a voting role in deliberations.

Specific duties and responsibilities will be outlined by charter prior to the start of the in-patient period of the study.

9.9 Data Management

Data management based on GCP refers to the activities defined to achieve safe routines to efficiently enter subject information into a database, avoiding errors.

The data management activities, such as procedures of validating data entered in the eCRF, will be described in a separate data management plan. The eCRF will be designed in accordance with the Clinical Study Protocol.

9.9.1 The web based eCRF

Clinical data (including AEs and concomitant medications) will be entered into a 21 CFR Part 11-compliant eCRF (Advantage eClinical Cloud) provided by [redacted] The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents, which are to be defined at the site before inclusion of the first subject.

Authorized study site personnel designated by the Investigator will complete data collection. Appropriate training will be completed with the Investigator and all authorized study site personnel prior to any data being entered into the system for any study subject. Each user will have a personal and unique account. eCRF data entries will be attributable to the unique user.

Captured data will be monitored electronically. Source data verification (SDV) and source data review will be performed at the site as described in the monitoring guidelines.

9.9.2 Entering of data into the eCRF

The eCRFs should always reflect the latest observations made during the subjects´ participation in the study. The eCRFs should be completed as soon as possible during or after the subject's visit.

If some assessments are not done, or if certain information is not available, not applicable or unknown, this will be indicated in the eCRF.

Entries triggering internal eCRF checks will be presented as errors and warnings. Forms with incomplete entries or that contains triggered errors cannot be set to completed state.

The Investigator must verify that all data entries in the eCRFs are accurate and correct by signing the completed eCRF.

Once all data have been entered, verified, and validated, the database will be locked.

After the eCRF has been locked, the data will be sent to the Sponsor and a copy to the research clinic to be filed in the Investigator Study File (ISF) for archiving.

9.9.3 The query process

The Monitor will review the eCRFs and source documents to evaluate them for completeness and consistency. The eCRF will be compared with the respective source documents to ensure that there are no discrepancies in critical data (SDV), in accordance with the monitoring guidelines.

Data Manager will perform data validation checks on data in the eCRF, as defined in the Data Management Plan (DMP).

Automated and/or Manual queries will be raised in the eCRF by Safety team, Study Monitor and/or by Data Manager respectively if inconsistencies or suspected errors are found. Queries shall be resolved, and data updated if applicable, in a timely manner by authorized study site personnel. The Monitor or Data Manager cannot enter or update data in the eCRF.

9.9.4 Source documents

Data entered into the subjects' medical record at the clinic and original analysis results at the laboratory will be considered source data. The eCRF is considered as source data when data is entered directly into the eCRF. A Data Management Plan (DMP) will be written, detailing the collection of data into the study database.

Source documents are all documents used by the Investigator or hospital that relate to the subject's medical history, that verifies the existence of the subject, the inclusion and exclusion criteria, and all records covering the subject's participation in the study. Source documents include laboratory results, memoranda, material dispensing records, subject files, etc.
The Investigator is responsible for maintaining source documents. These will be made available for inspection by the Monitor at each monitoring visit. Any supportive documentation submitted with the eCRF, such as laboratory or hospital records, should be clearly identified with the study ID and Subject Number. Any personal information, including name, should be removed or

rendered illegible to preserve individual confidentiality.

9.9.5 Audit trail

All changes made to data entered into the eCRF will be recorded in a protected audit trail (logging name of the person making the change, date and time, and if relevant the reason for change).

10 EMERGENCY PROCEDURES

10.1 Emergency contacts

In the case of a medical emergency the Investigator must contact the Medical Advisor at SBH (see below) without delaying care to the participant.

Name	Function in the study	Phone number and e-mail
[redacted]	[redacted]	[redacted]

10.2 Procedures in case of medical emergency

The Investigator is responsible for ensuring that there are procedures and expertise available to cope with medical emergencies during the study. The unit will be staffed 24/7 with medical staff and overnight with additional security staff. In the event that a participant requires a higher level of care than can be provided by the study staff on the inpatient unit, the study staff will follow the emergency procedures outlined in the MOP, which requires staff to provide emergency care and call 911. The PI will also be contacted as soon as possible and will alert the medical advisor.

11 STUDY MANAGEMENT

11.1 Changes in the approved study protocol

Any proposed change to the approved Final Clinical Study Protocol (including appendices) will be documented in a written and numbered protocol amendment. All amendments including substantial changes to the protocol must be submitted to appropriate IRB and Regulatory Authority for approval, according to applicable national regulations.

11.2 Study timetable

The end of the study is defined as the last follow-up contact of the last subject participating in the study.
The study is expected to start in Quarter 1, 2024 and it is expected be completed by Quarter 3 2024.

11.3 Discontinuation of the study

The SBH reserves the right to discontinue the investigation at any time but intends only to exercise this right for valid scientific or administrative reasons.

After such a decision, the Investigator must inform all participating subjects and perform relevant assessments, preferably according to the scheme for the final assessments. All delivered and unused test product and other study materials must be returned and all eCRFs completed as far as possible.

11.4 Reporting and publication of study results

A Clinical Study Report, in compliance with ICH E3; *Structure and content of Clinical Study Reports,* describing the conduct of the study, the statistical analysis performed and the results obtained for the primary and secondary endpoints of the study, will be prepared.

The study results will be reported in the clinicaltrials.gov database per applicable regulations within 12 months after completion of the study.

Results for the exploratory endpoints will be reported separately from the Clinical Study Report.

If the study duration exceeds one year, SBH must submit an annual safety report to the FDA and to the IRB (if applicable). The report will summarize all SAEs and contain an update of the risk-benefit evaluation if there has been any change since the approval of the clinical study.

Formal presentation or publication of data collected in this study should be considered as a joint publication by the Principal Investigator, other Investigators and a person appointed by SBH. Authorship will be determined by mutual agreement.

Before any publication (oral or written) of the results SBH will be given 30 days for review and comment on the manuscript. If the Principal Investigator and/or other Investigators have not submitted the results for publication within six months after completion of the final Clinical Study Report, the SBH has the right to publish. In this event, the Principal Investigator and/or other Investigators will be given 30 days to review and comment on the manuscript before it is submitted to a journal.

11.5 Disclosure and confidentiality

All unpublished information concerning the test product and research carried out by the SBH, including patent applications, manufacturing processes, scientific data from analysis of the primary and secondary endpoints etc., is considered confidential and the sole property of SBH. Scientific data resulting from the exploratory analyses is also confidential, but is the property of both SBH and the Principal Investigator / other Investigators. Disclosure to third parties must be limited to those undertaking legitimate peer review of the scientific and ethical aspects of the study and to those participating, including the recipients of test product, so that customary medical care and informed consent can be achieved.

11.6 Archiving

The Investigator must arrange for retention at the investigational site of a list of the subjects and their identifying code, subject files and other study documents. The archiving period must be adapted to regulations in force and should not be shorter than 15 years after the termination of the study and the present[[ation of the final clinical study report.

It is the responsibility of the SBH to inform the investigator/institution as to when these documents no longer need to be retained.

11.7 Insurance/indemnity

All subjects participating in the study will have insurance coverage by SBH, which is in line with applicable local laws and regulations.

11.8 Study agreements

The Principal Investigator must comply with all the terms, conditions, and obligations of the Clinical Trial Agreement (CTA) for this study.

Agreements between SBH and the study site must be in place before any study-related procedures can take place, or subjects be enrolled.

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13 APPENDICES

13.1 Signature pages

Principal Investigator

"I agree to the terms of this Study Protocol. I will conduct the study in accordance with the procedures specified in the protocol, the ethical principles in the latest version of the Declaration of Helsinki, ICH Good Clinical Practice and applicable regulatory requirements".

[redacted]		
Name	*Signature*	*Date*

Sponsor

"I agree to the terms of this Study Protocol."

Medical Advisor (Sponsor signatory)

[redacted]		
Name	*Signature*	*Date*

13.2 Declaration of Helsinki

http://www.up.ac.za/media/shared/Legacy/sitefiles/file/45/2875/declarationofhelsinki_fortaleza_brazil2013.pdf