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Clinical Study Protocol

Final, 3.0, dated 30-May-2018

valneva

Clinical Study Protocol

DRUG SUBSTANCE(S) VLA1701
VERSION NO. Final 3.0
STUDY CODE VLA1701-201
DATE 30-May-2018

RANDOMIZED DOUBLE-BLINDED PILOT STUDY CONFIRMING A HUMAN CHALLENGE MODEL USING LSN03-016011/A EXPRESSING LT AND CS17 AND INVESTIGATING THE SAFETY OF VLA 1701 (AN INVESTIGATIONAL ORAL CHOLERA AND ETEC VACCINE)

Phase 2 study

PROTOCOL NUMBER: **VLA1701-201**
IND NUMBER: **18106**

Sponsor

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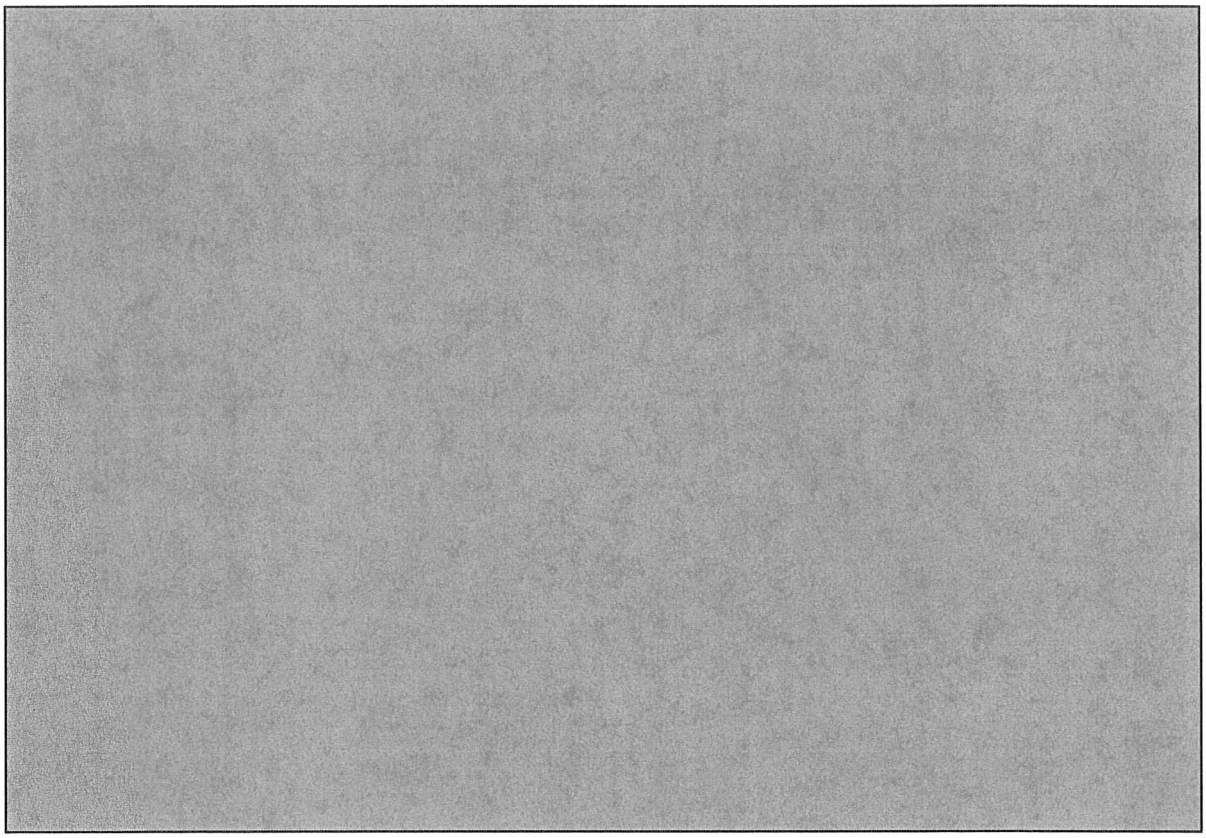
1. PROTOCOL SIGNATURE PAGE

Title of Clinical Trial: **RANDOMIZED DOUBLE-BLINDED PILOT STUDY CONFIRMING A HUMAN CHALLENGE MODEL USING LSN03-016011/A EXPRESSING LT AND CS17 AND INVESTIGATING THE SAFETY OF VLA 1701 (AN INVESTIGATIONAL ORAL CHOLERA AND ETEC VACCINE)**

Protocol Number: **VLA1701-201**

IND Number: **18106**

With their signature, Investigator and Sponsor agree to conduct this study in accordance with the Protocol, International Conference on Harmonization (ICH) and Good Clinical Practice (GCP) guidelines and with the applicable local regulatory requirements. Moreover, the site will keep all information obtained from the participation in this study confidential unless otherwise agreed in writing.



2. SERIOUS ADVERSE EVENT REPORTING

The Investigator will comply with applicable laws/requirements for reporting serious adverse events (SAEs) to the IRB. For information on the definition and assessment of adverse events (AEs), refer to Section 13.1.

All SAEs should be reported on the SAE Report Form and reported to Emmes through the Advantage eClinical data system or by a secure fax number within 24 hours after the Investigator has become aware of the event.

For details please refer to the VLA1701-201 Safety Monitoring Plan.

3. PREGNANCY REPORTING

The Investigator will comply with applicable laws/requirements for reporting pregnancies to the IRB. For information on the definition and assessment of pregnancies, refer to Section 13.8.

Pregnancies* should be reported on the Pregnancy Report Form and reported to Emmes through the Advantage eClinical data system or by a secure fax number within 24 hours after the Investigator has become aware of the event.

For details please refer to the VLA1701-201 Safety Monitoring Plan

*A pregnancy is not considered an SAE. If a seriousness criterion applies in addition to the pregnancy (e.g. hospitalization, congenital anomaly/birth defect) the pregnancy qualifies as an SAE. In such case a Pregnancy Report Form and an SAE Report Form have to be filled out.

4. CLINICAL STUDY SYNOPSIS

INVESTIGATIONAL PRODUCT, DOSAGE AND MODE OF ADMINISTRATION	
Name of Investigational Medicinal Product (IMP)	• VLA1701 • Placebo • Challenge Strain: E. coli strain LSN03-016011/A (LT+, ST-, CS17)
Name(s) of Active Ingredient(s)	**VLA1701** is an oral inactivated vaccine containing inactivated *V. cholerae* bacteria as well as rCTB: + $1.25x10^{11}$ vibrios of the following strains: › *Vibrio cholerae* O1 Inaba EL Tor strain (formalin inactivated) $6.25x\ 10^{10}$ vibrios › *Vibrio cholerae* O1 Ogawa classical strain (heat inactivated) $6.25x\ 10^{10}$ vibrios Recombinant cholera toxin B subunit (rCTB) 1mg
This is a single-center, double-blind, placebo-controlled, Phase II vaccination and challenge study designed to confirm a human challenge model with E. coli strain LSN03-016011/A (LT+, ST-, CS17), as well as collect expanded safety and immunogenicity data.	
CLINICAL CONDITION(S)/INDICATION(S)	
Active immunization for the prevention of disease caused by ETEC	
Study Phase	Phase 2
PLANNED STUDY PERIOD	
Initiation	Planned Q2/2018
Duration	The maximum overall study duration (study start (i.e. First Subject In) to end of study (i.e. Last Subject Last Visit) is estimated to be ~7-8 months.
Completion	Last 6 months safety call is expected to occur in November 2018 An Interim Analysis study will be performed after subjects have completed the Inpatient Period (Visit 12) and the final analysis will be performed once the last subject has completed the study, i.e. Month 6 (Visit 14). A clinical study report will be compiled upon availability of the final statistical results.

STUDY PURPOSE

The purpose of this study is to confirm the characteristics of the ETEC challenge model for LSN03-016011/ A, an LT and CS17 expressing ETEC strain.

STUDY OBJECTIVES

Primary Objectives

- The primary objective of this study is to evaluate the number of subjects with moderate to severe diarrhea after challenge with ETEC strain LSN03-016011/ A.

Secondary Objectives

- To assess the scale of disease severity following Human Challenge with ETEC strain LSN03-016011/ A as described by Porter, et al; An Evidenced-Based Scale of Disease Severity following Human Challenge with Enteroxigenic Escherichia coli; Plos one, 2016.
- To assess the safety of VLA1701 in a healthy adult population aged 18 to <50 years.

STUDY DESIGN

Investigator and sites	Center for Immunization Research, Baltimore, MD USA
Study participants	Up to 34 healthy subjects aged 18 to <50 years will be enrolled and randomized 1:1 to either receive VLA1701 or placebo.

Product	N	Dose
VLA1701	17	2 doses about 1 week apart
Placebo	17	2 doses about 1 week apart
Challenge	30	approximately $5x10^9$ cfu of ETEC strain LSN03-016011/ A

Study Type	Double-blind, placebo-controlled, randomized
Control Type	Placebo
Study Indication Type	Prevention
Blinding Scheme	Double Blind

Study Design

This is a single-center, double-blind, placebo-controlled, Phase II vaccination and challenge study designed to confirm a human challenge model with E. coli strain LSN03-016011/A (LT+, ST-, CS17), as well as collect expanded safety and immunogenicity data.

The study will be carried out in two phases:

Vaccination phase: up to 34 subjects will be randomized 1:1 to receive 2 doses of either VLA1701 or placebo orally. The doses will be given 7 days apart and subjects will be followed as an outpatient for safety.

Challenge Phase: 30 Subjects, out of the 34 subjects, will be challenged (see section 9.4). In the morning, after approximately 90 minutes of fasting, 30 subjects will ingest 120 ml of USP sodium bicarbonate solution (13.35 g/liter) followed in 1 minute by another 30-ml bicarbonate solution containing the challenge strain. Subjects continue to fast for 90 min following challenge.

After challenge, subjects will be monitored for diarrhea and other signs/symptoms of enteric illness by daily medical checks, vital sign determinations, grading and weighing of all stools.

Five days after challenge, or sooner if subjects meet early treatment criteria, subjects will be treated with antibiotics. Subjects will be discharged after at least 2 doses of antibiotic treatment, clinical symptoms are resolved or resolving and the subject has produced two stool samples that were negative for LSN03-016011/A by microbiological culture. All subjects will be followed up with an in-person visit at Day 44 after vaccination (i.e., 28 days after challenge); a telephone call to check for any serious medical conditions, new onset of chronic illnesses and functional bowel disorders will be scheduled approximately 6 months after their first vaccination.

The overall study design is displayed in Figure 1 below.

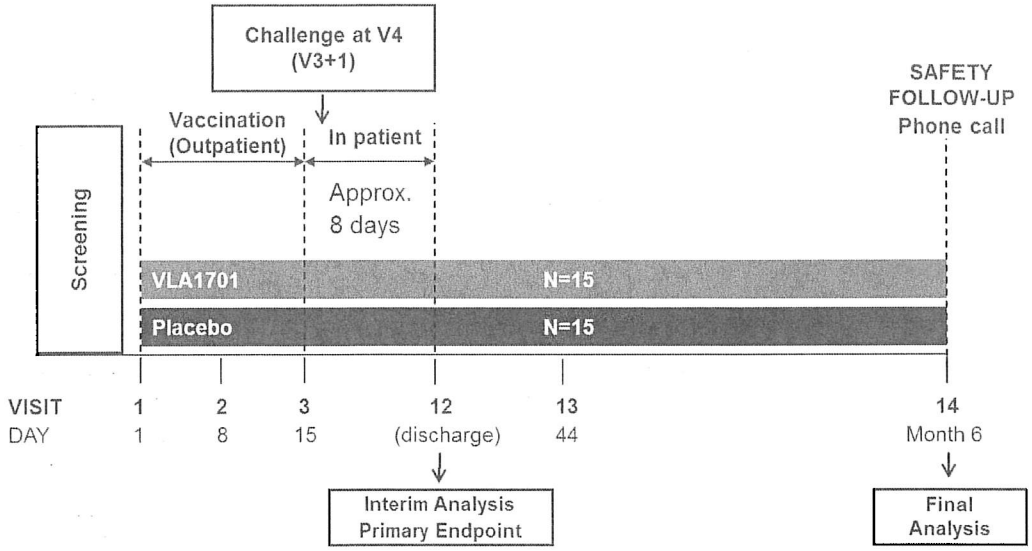


Figure 1: Overall Clinical Phase 2 Study Design

SUBJECT ENROLMENT - RANDOMIZATION

Subjects will be randomized in a 1:1 ratio to either receive VLA1701 or placebo. An unblinded statistician at Assign Data Management and Biostatistics will prepare a randomization list. Actual randomization of eligible subjects will be performed by site staff using the enrollment module of Advantage eClinical.

STUDY ENDPOINTS

Primary Endpoint

- Percentage of subjects with severe to moderate diarrhea within 120 hours of challenge with ETEC strain LSN03-016011/A.

The Definition of diarrhea is as follows:

During the inpatient period, each stool passed is collected, weighed, and graded as follows: grade 1, firm formed; grade 2, soft formed; grade 3, viscous opaque liquid or semiliquid which assumed the shape of the container; grade 4, watery opaque liquid; and grade 5, clear watery or mucoid liquid.

Stools defined as grade 3, 4, or 5 are considered to be loose and to potentially contribute to an episode of diarrhea:

- Severe diarrhea: at least six loose stools or > 800 g in 24 h;
- Moderate diarrhea: 4-5 loose stools or 401 to 800 g in 24 h;
- and mild diarrhea: 1-3 loose stool or ≤ 400 g in any 24-hour period.
- In calculating the total number and weight of diarrheal stools following challenge only stools which contributed to an episode of diarrhea according to these definitions will be included.
- An episode of diarrhea will be considered complete after 24 hours without a loose stool.

Secondary Endpoints

Severity of disease induced after challenge with ETEC strain LSN03-016011/ A

- Calculation of disease severity score after challenge with ETEC strain LSN03-016011/ A

Safety of VLA 1701

- Percentage of subjects with solicited adverse events within 7 days after each vaccination.
- Percentage of subjects with any adverse events (AEs) observed up to Visit 4 (before Challenge) and during the entire study period (including clinically significant laboratory parameter changes).
- Percentage of subjects with serious adverse events (SAEs) observed up to Visit 4 (before Challenge) and during the entire study period.
- Percentage of subjects with any IMP-related AEs observed up to Visit 4 (before Challenge) and during the entire study period (including clinically significant laboratory parameter changes).

- Percentage of subjects with IMP-related SAEs observed up to Visit 4 (before Challenge) and during the entire study period.

Exploratory Endpoints:

Disease induced after challenge with ETEC strain LSN03-016011/ A

- To quantify the number and quality of loose stools and grade the intensity of symptoms occurring after challenge.
- Number and percentage of subjects with diarrhea of any intensity.
- Number and percentage of subjects with diarrhea of any intensity, based on evaluation of stool volume only.
- Time to onset of diarrhea from the time of receiving the ETEC challenge (incubation period).
- Time, in hours, from the first diarrheal stool to the last diarrheal stool (duration of diarrhea).
- Mean total weight of grade 3-5 stools passed per subject.
- Mean number of grade 3-5 stools per subject.
- Total mass/volume of diarrheal stools.
- Maximum 24-hour stool output (volume and frequency).
- Number and percentage of subjects requiring early intervention with antibiotic therapy due to the severity of diarrhea.
- Number and percentage of subjects with fever, nausea, bloating, vomiting, myalgia, arthralgia, abdominal pain, abdominal cramping, malaise, headache, light headedness.
- Number of colony forming units (cfu) of the challenge strain per gram of stool on days 2 and 4 after challenge (V6 and V8).
- Number and percentage of subjects requiring IV fluids.
- Number and percentage of subjects developing moderate to severe diarrhea by ABO blood type.

Immunogenicity of VLA-1701:

- Systemic immune responses after two doses of VLA1701 at 7 days post vaccination.
- Mucosal immune responses after two doses of VLA1701 at 7 days post vaccination.
- Systemic immune responses after challenge with ETEC strain LSN03-016011/A at 7 days and 1 month after challenge.
- Mucosal immune responses up to 1 month after challenge with ETEC strain LSN03-016011/A at 7 days and 1 month after challenge.

CRITERIA FOR INCLUSION / EXCLUSION

Approximately 34 adults of both genders who satisfy the inclusion and exclusion criteria listed below will be invited to participate in the study.

Inclusion Criteria

Subjects who meet **ALL** of the following criteria are eligible for this study:

1. Healthy male and non-pregnant female subjects aged 18 to <50 years; health status is assessed by investigator at time of screening based on medical history, physical examination, and laboratory parameters.
2. BMI of 19.0 to 35.0 kg/m2
3. Willingness to participate after informed consent has been obtained from the subject prior to any study related procedures.
4. Completion of a training session and demonstration of comprehension of the protocol procedures and knowledge of ETEC-associated illness by passing a written examination.
5. If subject is of childbearing potential:
    a) Negative pregnancy test at screening (Visit 0) with understanding to not become pregnant within 28 days after challenge;
    b) Subject has practiced an effective method of contraception (see below) during the 30 days before screening (Visit 0);
    c) Subject agrees to employ adequate birth control measures for the duration of the study. This includes one of the following measures:
        - Hormonal contraceptives (e.g. implants, birth control pills, patches);
        - Intrauterine device;
        - Barrier type of birth control measure (e.g. condoms, diaphragms, cervical caps);
        - Vasectomy in the male sex partner ≥3 months prior to first vaccination.

Exclusion Criteria

Subjects who meet **ANY** of the following criteria are **NOT** eligible for this study:

1. Participated in research involving investigational product within 30 days before planned date of first vaccination or planned use through Day 44;
2. Any prior exposure to ETEC (including LSN03-016011/A) or cholera occupationally or received LT (Or any mutant forms of LT (e.g., LTR192G, LTR192GL211A), ETEC, or cholera vaccine);
3. Subjects with known abnormal stooling patterns (fewer than 3 per week or more than 3 per day);
4. Known allergies to any component of the vaccine;

5. Subjects with known allergies to more than 1 planned antibiotics: Ciprofloxacin, Amoxicillin, trimethoprim-sulfamethoxazole;
6. History of diarrhea while traveling in a developing country within the last 3 years;
7. Subjects whose occupation involves handling of ETEC or cholera bacteria;
8. Women who are pregnant or breastfeeding;
9. Significant medical conditions including chronic, immunosuppressive, malignant, or gastrointestinal diseases (e.g. History of Irritable Bowel Syndrome (as defined by the Rome III criteria or medical diagnosis) or gastric ulcer disease) or enteric, pulmonary, cardiac, liver or renal disease. Some medical conditions which are adequately treated and stable may be acceptable in the study (e.g. hypertension);
10. Significant abnormalities in screening lab hematology or serum chemistries, as determined by PI or PI in consultation with the independent Research Monitor and sponsor;
11. Use of any medication known to effect the immune system (e.g. systemic corticosteroids) within 30 days of vaccination or planned use during active study period (excluding inhaled steroids);
12. Evidence of confirmed infection with HIV, Hepatitis B or Hepatitis C;
13. Subjects with IgA deficiency (serum IgA < 7 mg/dl or limit of detection of assay);
14. Regular use of antacids, antidiarrheal, loperamide, bismuth subsalicylate, diphenoxylate or similar medication less than 2 weeks prior to enrolling in the study and through the inpatient portion of the study;
15. Known or suspected alcohol abuse or illicit drug use within the last year, positive urine toxicology for opioids, benzodiazepines or amphetamines;
16. Persons who are committed to an institution (by virtue of an order issued either by the judicial or the administrative authorities);
17. Persons who are in a dependent relationship with the sponsor, an investigator or other study team members, or the study center. Dependent relationships include close relatives and household members (i.e. children, partner/spouse, siblings, parents) as well as employees of the investigator or study center personnel;
18. 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.

STATISTICAL ANALYSIS

Sample Size Justification

Up to 34 subjects will be randomized 1:1 to receive either VLA1701 or placebo, 30 subjects will be challenged.

There was no formal sample size calculation for this pilot study. Sample size was planned considering site capabilities and reference of studies previously described for challenge studies. The primary objective is to gain information on disease induced by the challenge strain in the placebo group in order to confirm published characteristics of the challenge

strain and to appropriately design future studies based on the findings of this pilot study (e.g. if 7 or more subjects in Placebo group develop diarrhea, a diarrhea rate induced by the challenge strain of 70% or above cannot be excluded with one-sided 95% CI).

Statistical Methods

The primary analysis will be evaluated in the challenge population (i.e. all subjects who received two doses of the vaccine and the challenge dose). ETEC disease-specific events (including severe to moderate diarrhea after challenge for the primary endpoint) will be reported as "ETEC Disease-specific Expected Events" (as defined in section 12), after challenge (Visit 4) up to discharge from inpatient period (Visit 12). These events will not be considered adverse events but will be reported separately in number and percentages for the inpatient period Visit 4 to Visit 12 (i.e. up to 7 days after challenge) and will be compared between the study arms using Fisher's exact test.

Disease severity score will be calculated as described in Porter, et al; An Evidenced-Based Scale of Disease Severity following Human Challenge with Enterotoxigenic Escherichia coli; Plos one, 2016. Differences in the disease scores between groups will be compared using a Student's t-test with a 2-sided alpha = 0.05.

All subjects entered into the study, who receive at least one dose of VLA1701, will be included in the safety analysis. The number and percentage of subjects with solicited adverse reactions and unsolicited AEs and SAEs up to 7 days after each vaccination, during the inpatient period as well as during the entire study period (i. e. including Visit 13 (1 month after challenge) and Visit 14 (6 month after first vaccination)), will be presented for each study arm overall and by body system / preferred term and will be compared using Fisher's exact test.

Changes in laboratory values and the frequency of clinically relevant abnormal values will be analyzed descriptively.

The exploratory immunogenicity analysis will be done on collected Serum, PBMCs and stool samples during the study. The following assays will be conducted:

- Serum vibriocidal titer at baseline, 7 days after vaccination, 7 days after challenge and 1 month after challenge
- Serum IgA and IgG against LT/CT at baseline, 7 days after vaccination, 7 days after challenge and 1 month after challenge
- Serum IgA and IgG against CS17 at baseline, 7 days after vaccination, 7 days after challenge and 1 month after challenge
- ALS IgA against LT/CT and against CS17 at baseline, 7 days after vaccination, 7 days after challenge and 1 month after challenge
- Fecal total IgA at baseline, 7 days after vaccination, 7 days after challenge and 1 month after challenge
- Fecal IgA against LT/CT and against CS17 at baseline, 7 days after vaccination, 7 days after challenge and 1 month after challenge

Data Analysis

All tests and CIs will be two-sided unless stated otherwise. Evidence of statistically significant differences of endpoints will require a p-Value of 0.05 or less in a two-sided comparison.

An interim analysis including the primary endpoint analysis will be performed after all subjects have gone through challenge and have been released from the inpatient facility (Visit 12). A final analysis will be conducted once the last subject has completed the study, i.e. after the Safety Follow-up phone call at Month 6.

A Clinical Study Report will be written after the final analysis, once all data are analyzed.

ASSESSMENT OF SAFETY PARAMETERS

Clinical Monitoring

After vaccination, subjects will have memory cards that allow them to capture symptoms and adverse events that occur within 7 days after vaccination.

After challenge, subjects will be monitored for expected events associated with ETEC illness (e.g. diarrhea and other signs/symptoms of enteric illness) by daily medical checks, vital sign determinations, grading and weighing of all stools.

Subjects will be discharged after at least 2 doses of antibiotic treatment, clinical symptoms are resolved or resolving and the subject has produced two stool samples that were negative for LSN03-016011/A by microbiological culture. All subjects will be followed up with an in-person visit (Visit 13); a telephone call to check for any serious emergent medical conditions, new onset of chronic illnesses and functional bowel disorders will be scheduled approximately 6 months after their first vaccination (Visit 14). At that point, the Rome III survey for functional bowel disease (Irritable Bowel Syndrome (IBS) survey) will be administered.

Clinical Management

1. Fluid Management

- Oral: Any subject passing a grade 3-5 stool will be encouraged to start drinking liquids (e.g. Gatorade or ORS) at a rate equal to their stool output.
- Intravenous: A subject may be administered IV fluids if determined necessary by the study physician, i.e., diarrhea with nausea/vomiting and unable to orally keep up with output, hypovolemia, or other reason.

2. Antibiotic Treatment

All challenge study subjects will be treated with ciprofloxacin (or trimethoprim-sulfamethoxazole or amoxicillin) after 120 hours post-challenge, or earlier if disease severity warrants intervention (criteria outlined below).

During the challenge phase of the trial, a subject will qualify for early treatment (< 120 hours [5 days] after challenge) with ciprofloxacin (or trimethoprim-sulfamethoxazole or amoxicillin) if any of the following criteria are met post-challenge:

- Severe diarrhea (6 or more loose stools or > 800g in 24 hours)
- Stool output consistent with moderate diarrhea for 48 hours
- Mild or moderate diarrhea and 2 or more of the following symptoms: severe abdominal pain, severe abdominal cramps, severe nausea, severe headache, severe myalgia, any fever (≥ 38.0°C), or any vomiting
- A study physician determines that early treatment is warranted for any reason.

Discharge Procedures

Subjects will be discharged from the inpatient phase of the study at approximately 8 days after challenge when clinical symptoms are resolved or resolving, at least two doses of antibiotics have been taken AND two consecutive stool cultures are negative for the challenge strain.

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6. LIST OF ABBREVIATIONS

Abbreviation	Explanation
abx	antibiotic
AE	Adverse event
ALS	Antibody Lymphocyte Supernatant
ALT	Alanine aminotransferase
ATC	Anatomical therapeutic chemical
B/P	Blood Pressure
BS	Bile Salts
BMP	Basic Metabolic Panel
C	Celsius
CBC	Complete blood count
CDAD	*Clostridium difficile*-associated diarrhea
CEC	Clinical Endpoint Committee
CFR	Code of Federal Regulations
CF	Colonization Factor
CFA	Colonization Factor Antigen
CFU	Colony Forming Unit
CIR	Center for Immunization Research
CI	Confidence Interval
CRA	Clinical Research Associate
CRO	Contract Research Organization
CT	Cholera toxin
rCTB	recombinant Cholera Toxin B subunit
cGMP	Current Good Manufacturing Practice
cm	Centimeter
ECG	Electrocardiogram
eCRF	Electronic case report form
EEA	European Economic Area
EDC	Electronic data capture
e.g.	For example
ELISA	Enzyme-linked immunosorbent assay
ETEC	Enterotoxigenic E coli
EU	European Union
F	Fahrenheit
FDA	US Food and Drug Administration
F/U	Follow Up

g	Gramm
GCP	Good Clinical Practice
GI	Gastrointestinal
GMP	Good Manufacturing Practice
HBsAG	Hepatitis B surface antigen
HIV	Human immunodeficiency virus
HR	Heart Rate
IB	Investigator's Brochure
IBS	Irritable Bowel Syndrome
ICF	Informed consent form
i.e.	Id est/ that is
ICH	International Conference on Harmonization
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IMP	Investigational Medicinal Product
IND	Investigational New Drug
I PD	Intermediate Process Development
IRB	Institutional Review Board
IVF	Intravenous Fluids
JHSPH	Johns Hopkins Bloomberg School of Public Health
LPS	Lipopolysaccharide
LT	Labile toxin
MAC	MacConkey
MAH	Marketing Authorization Holder
MCB	Master Cell Bank
µg	Microgram
mL	Milliliter
MSA	Mannitol Salt Agar
NAMRU-3	Naval Medical Research Unit-three
NCS	Not clinically significant
NMRC	Naval Medical Research Center
ORS	Oral Rehydration Solution
PBF	WRAIR Pilot Bioproduction Facility
PCB	Production Cell Bank
PCR	Polymerase chain reaction
PBMC	Peripheral blood mononuclear cell

PI	Principal Investigator
PI-IBS	Post-Infectious Irritable Bowel Syndrome
RS MCB	Research Seed Master Cell Bank
SAE	Serious adverse event
SIC	Subject Identification Code
SOP	Standard operating procedure
SSP	Study-specific procedure
ST	Stable toxin
SUSAR	Suspected Unexpected Serious Adverse Reaction
TSA	Tryptone Soy Agar
TSB	Tryptone Soy Broth
VS	Vital Signs
WBC	White blood count
WC	Whole-cell
WHO	World Health Organization
WRAIR	Walter Reed Army Institute of Research
XLD	Xylose Lysine Deoxycholate

7. INTRODUCTION

7.1 Pharmacological Class

VLA1701 is an oral killed whole-cell (WC) vaccine candidate, under investigation for protection against cholera caused by Vibrio cholerae O1 (classical and El Tor biotypes) and diarrhea caused by LT-producing enterotoxigenic E. coli (ETEC).

For further details please refer to the Investigator's Brochure.

7.2 Targeted Indications

VLA1701 is intended for the prevention of and protection against cholera and ETEC-producing heat-labile enterotoxin (LT)

- Cholera: Adults and children from 2 years of age who will be visiting areas with an ongoing or anticipated epidemic or who will be spending an extended period of time in areas in which cholera infection is a risk.
- ETEC-diarrhea: Adults and children from 2 years of age who will be visiting areas posing a risk of diarrheal illness caused by LT-producing ETEC.

7.3 Background on Cholera & ETEC

Diarrheal disease is a leading global health problem. It has been estimated that it causes 4% of all deaths and 5% of health loss to disability. It is most commonly caused by gastrointestinal (GI) infections which kill around 1.3 million people globally each year, mostly children in developing countries (Global Burden of Disease 2015 study Lancet, 2016). About 80% of these diarrheas are caused by bacteria that produce one or more enterotoxins (Connor, 2014; Jelinek & Kollaritsch, 2008). Cholera, resulting from infection with V. cholerae bacteria, is the most severe of these diseases, while infection with enterotoxigenic Escherichia coli (ETEC) causes the largest number of cases (Kollaritsch & Wiedmann, 2007). The total number of cholera cases annually is uncertain since several affected countries do not monitor and/or underreport the disease. In 2011 alone, the WHO reported a total of 589,854 cases of cholera from 58 countries, including 7,816 deaths (World Health Organization, 2012). However, the true global disease burden is estimated to be 3 to 5 million cases and up to 120,000 deaths per year (World Health Organization, 2008; Zuckerman, Rombo & Fisch, 2007; Clemens et al, 2008). This discrepancy results from underreporting and other surveillance system limitations, including inconsistencies in case definition and lack of standard vocabulary. Some countries report laboratory confirmed cases only, while many more cases correspond to the WHO standard case definition. Cases of cholera reported to WHO do not include the numerous cases labeled as acute watery diarrhea (AWD) in several countries in Africa and Central and South-East Asia. Among the cholera cases developing symptoms, 80% of episodes are of mild or moderate severity (World Health Organization, 2012). Similarly, since most ETEC infections are mild, their total number is difficult to assess, but diarrhea caused by ETEC has been estimated to account for 210 million episodes and approximately 400,000 deaths per year (Jelinek & Kollaritsch, 2008). ETEC is also the single most common cause of traveler's diarrhea (Wkly Epidemiol Rec, 2008).

Diarrhoea in travelers can result from a variety of intestinal pathogens with bacterial pathogens being the predominant risk, accounting for 80%–90% of cases. Enterotoxigenic Escherichia Coli (ETEC), followed by Campylobacter jejuni, Shigella spp., and Salmonella spp. are the most common pathogens (Prevention, C.f.D.C.a., 2016).

Specific virulence factors such as enterotoxins and colonization factors differentiate ETEC from other categories of diarrhoeagenic E. coli. The ETEC pathogenesis suggests that the organism colonize the small intestine by virtue of colonization factors, followed by the elaboration of heat-stabile (ST) and / or heat labile (LT) toxin.

Secretory watery diarrhoea can be caused by either LT or ST produced by ETEC, both of which stimulate chloride secretion and inhibit chloride absorption in small intestine epithelial cells. LT is structurally and functionally similar to cholera toxin. ST is a very small protein (only 19 amino acids) that does not appear to elicit an immune response. Colonization factors (CFs) are proteinaceous surface polymers that facilitate adherence to the intestinal mucosa. At least 25 different CFs have been described to date in human ETEC strains and most are plasmid-encoded. The role of different colonization factors in determining severity of illness is poorly understood (Qadri, et al., 2005).

The heat labile (LT) toxin is structurally, patho-physiologically and immunologically similar to cholera toxin. The quaternary structure of LT and CT are also almost identical, each with one A subunit surrounded by a pentamer of B subunits, which bind equally well to the GM1 ganglioside receptor (Long, et al, 2008; Flores et al, 2008). Most antibodies induced by CT are directed against the B subunit, (Clemens et al, 1988; Long et al, 2010) and CTB-specific antibodies are able to cross-react with LT (Clemens et al, 1988). It has been shown that sera from individuals vaccinated with CT were able to neutralize LT, (Long et al, 2010) and that individuals infected with V. cholera, through challenge or natural infection, showed an increase in cross-reacting anti-toxin antibodies, suggesting similar antigenic characteristics of both toxins (Clemens et al, 1988).

Immune response to the cholera toxin B subunit has been shown to also neutralise the toxic effect of the ETEC heat labile toxin.

7.4 Investigational medicinal product: VLA1701

The investigational medicinal product VLA1701 used for the pilot human challenge trial VLA1701-201 is an oral inactivated vaccine containing inactivated V. cholerae bacteria as well as rCTB.

+ A total of $1.25x10^{11}$ vibrios of the following strains:
    - › Vibrio cholerae O1 Inaba EL Tor strain (formalin inactivated) $6.25x\ 10^{10}$ vibrios
    - › Vibrio cholerae O1 Ogawa classical strain (heat inactivated) $6.25x\ 10^{10}$ vibrios
+ Recombinant cholera toxin B subunit (rCTB) 1 mg

7.4.1 VLA1701 vaccine dose and justification

VLA1701 represents a [redacted]. The formulation of [redacted] is shown below:

+ A total of $1.25x10^{11}$ bacteria of the following strains:
    - › Vibrio cholerae O1 Inaba, classical biotype (heat inactivated) $31.25x10^{9}$ vibrios

› Vibrio cholerae O1 Inaba, El Tor biotype (formalin inactivated) $31.25x10^9$ vibrios

› Vibrio cholerae O1 Ogawa, classical biotype (heat inactivated) $31.25x10^9$ vibrios

› Vibrio cholerae O1 Ogawa, classical biotype (formalin inactivated) $31.25x10^9$ vibrios

+ Recombinant cholera toxin B subunit (rCTB) 1 mg

Although, the overall bacterial count of whole cell killed vibrios remains the same, VLA1701 represents a change of formulation from Dukoral as it only contains two – instead of four- different whole cell inactivated vibrio components; formalin inactivated Vibrio cholerae O1 Inaba EL Tor strain and heat inactivated Vibrio cholerae O1 Ogawa classical strain. [redacted]

VLA1701 contains an increased number of V. cholerae O1 bacteria of biotype El Tor since this is the causative organism of the seventh cholera pandemic that started in 1961. During the sixth pandemic all isolates were of the classical biotype. Both Ogawa and Inaba strains are included in line with the requirements for parenteral cholera vaccines as set forth in e.g. 21CFR 620.30 and Eur. Pharm. 1997:0154. The El Tor strain is formalin inactivated [redacted]

7.4.2 Dukoral vaccine

Dukoral is an oral killed whole-cell (WC) vaccine protecting against cholera caused by Vibrio cholerae O1 (classical and El Tor biotypes) and diarrhea caused by LT-producing enterotoxigenic E. coli (ETEC).

Dukoral was introduced in Sweden in December 1991, approved in the EU (including Norway and Iceland) through a centralized procedure in April 2004, and is registered in the European Economic Area (EEA) and in 35 countries outside Europe. Dukoral is on the World Health Organization (WHO) list of pre-qualified vaccines and obtained WHO registration in 2001.

The vaccine is administered orally in 2 doses at least 1 week apart. The vaccine is provided as 2 components, a. active substance in a glass vial and b. effervescent granules contain sodium hydrogen carbonate buffer in a sachet. The effervescent granules are dissolved in a glass of water before adding the component from the glass vial. The vaccine is to be consumed in the presence of the buffer in order to protect the antigen from the acidic components in stomach.

Dukoral consists of killed V. cholerae and the non-toxic recombinant cholera toxin B subunit. The vaccine acts locally in the gastrointestinal tract to induce an IgA antitoxic and antibacterial response (including memory) comparable to that induced by cholera disease itself. The protection against cholera is specific for both Inaba and Ogawa serotypes and El Tor and Classical biotypes. O-antigens as well as toxin B subunit will induce immunity. The vaccine confers protection against cholera, as well as LT-producing ETEC; either LT alone or both LT and ST together.

The vaccine was first licensed in Sweden in 1991 and then later in other countries, including Canada and Australia. In 2004 the vaccine was licensed by the European Medicines Agency (EMA) for use in Europe. Currently the vaccine is licensed and marketed in approximately 15 territories around the world.

7.4.2.1 Efficacy

Efficacy of Dukoral against cholera and ETEC was assessed in three randomized double-blind placebo-controlled clinical trials conducted in Bangladesh (endemic region, Study 6) and in Peru (non-endemic region, Studies 27 and 30), with a reduction in disease episodes of 85% in the first 6 months and 86% in the first 3 months respectively. A protection rate of 84% was shown in a mass vaccination campaign using Dukoral in an endemic neighborhood of Beira, Mozambique, a setting with high human immunodeficiency virus (HIV) prevalence (Study 49). In a mass vaccination campaign in Zanzibar, Dukoral conferred 79% direct protection against cholera in those subjects who received two doses (Study 50). In this study, herd protection was suggested by the statistically significant inverse relation between vaccine coverage and cholera risk.

7.4.2.2 Safety

The safety of Dukoral was assessed in clinical trials, including both adults and children from 2 years of age, conducted in endemic and non-endemic countries for cholera and enterotoxigenic Escherichia coli (ETEC) producing heat-labile enterotoxin (LT).

The clinical development for Dukoral was initiated more than 30 years ago. Since that time, Dukoral has been included in Company-sponsored trials. However, due to the maturity of the product in terms of its clinical development, a reliable estimate of subject exposure in all clinical trials with Dukoral is not available. The subject exposure in 4 pivotal clinical trials for Dukoral is described below.

Overall, an estimated 98,344 healthy subjects have been enrolled in the Dukoral pivotal clinical trials, of which approximately 59,910 subjects have received Dukoral (see Table 1). Table 2 shows exposure in pivotal clinical trials by country and age.

Evaluation of safety varied between trials with respect to mode of surveillance, definition of symptoms and time of follow-up. In the majority of studies adverse events were assessed by passive surveillance.

No serious adverse events have been reported in clinical trials with Dukoral since development. The most frequently reported adverse reactions, such as gastrointestinal symptoms including abdominal pain, diarrhea, loose stools, nausea and vomiting, occurred at similar frequencies in vaccine and placebo groups.

Table 1: Estimated cumulative subject exposure from pivotal clinical trials

Study Number Country	CTB rCTB	Indication	Schedule	Age range (years)	Number of Subjects[a]			
					Total	Dukoral (Oral Cholera Vaccine)		Placebo
						BS-WC	WC	
Study 6 Bangladesh	CTB	Cholera		2 to 5	11,630	3,797	3,951	3,882
				6 to 10	14,969	5,028	4,887	5,054
				11 to 15	12,582	4,159	4,276	4,147
				>15	24,317	8,157	8,023	8,137
Study 27 Peru	rCTB	Cholera		16 to 48	1,426		710	716
Study 30 Peru	rCTB	Cholera	2 doses	2 to 5	2,368	1,198		1,170
				6 to 15	6,782	3,436		3,346
				15 to 65	8,649	4,378		4,271
			3 doses	2 to 5	2,040	1,040		1,000
				6 to 15	6,058	3,065		2,993
				15 to 65	6,908	3,498		3,410
Study 6 Bangladesh	CTB	ETEC	≥2 doses	2 to 15, women >15	49,612[b]	24,842	24,770	0
Study 9 Morocco	CTB	ETEC	2 doses	tourists travelling to Morocco	615	307		308

Key: BS-WC=B subunit whole-cell vaccine; CTB=cholera toxin B subunit; ETEC=enterotoxigenic *Escherichia coli*; rCTB=recombinant cholera toxin B subunit; WC=killed whole-cell vaccine

Table 2: Subject exposure from completed pivotal clinical trials by country/ area and age

Treatment	Number of Subjects[a]
Dukoral	59,910
Comparator	0
Placebo	38,434

a. Based on per protocol exposure data from completed pivotal clinical trials

7.4.2.3 Post-Marketing-Safety Data

7.4.2.3.1 Post-Marketing Studies

In the period from April 2006 to April 2007 a post-marketing safety surveillance study was performed (DUK-PMS06) to answer the question if the experience of diarrhea among travelers vaccinated with Dukoral influences the travelers' willingness to be vaccinated again (Gulliksen et al, 2007). A total of 297 subjects filled in an anonymous questionnaire, of which 33 subjects reported at least one adverse event. Of the 33 reports, 28 were considered related and five were considered unrelated. There were no SAEs. The most frequently reported adverse events were nausea followed by stomach pain and diarrhea or soft stool. Of the subjects experiencing diarrhea only 12% stated that they were dissatisfied with the vaccine compared to 5% among those who did not have diarrhea.

7.4.2.4 Worldwide Market Authorization Status and Subject Exposure

Dukoral has been licensed in Sweden since 1991 containing the chemically purified CTB component, and since 1992 with the recombinant B-subunit, rCTB.

Dukoral has been approved as a vaccine for immunization against cholera centrally in the EEA and in addition in 35 countries outside Europe. In most of the countries, it is also indicated for immunization against ETEC (exceptions: Australia, EEA, Hong-Kong, Indonesia, South Korea, and United Arab Emirates).

Dukoral is on the WHO list of pre-qualified vaccines and obtained WHO registration in 2001.

A crude estimate of the number of people vaccinated with Dukoral can be calculated from the total sales volumes and the number of doses recommended per vaccination. Over 20 million doses of Dukoral have been sold since first registration in 1991 until the end of April 2014. Assuming that each patient received two doses of Dukoral, approximately 7.8 million immunizations worldwide can be estimated during that time period. However, the number of effectively vaccinated subjects might be lower because Crucell (now known as Valneva Sweden AB) has no information on the number of vaccinated children (three doses), or doses sold as booster doses to adults and especially to children for cholera and ETEC protection which need to be taken every 6 or 3 months, respectively.

7.4.2.5 Spontaneously Reported Post-Marketing Adverse Drug Reactions (ADRs)

In the period from 1991 up to 28 April 2014, a total of 2,575 adverse events including 456 SAEs were spontaneously reported to Crucell Sweden (now known as Valneva Sweden AB). Overall, the spectrum of spontaneously reported adverse reactions following use of Dukoral has been similar to that known from clinical studies.

The most frequently reported spontaneous adverse reactions were GI symptoms (such as diarrhea, vomiting, nausea, abdominal pain, flatulence, and abdominal discomfort), pyrexia, headache, dizziness, chills, malaise, fatigue, arthralgia, urticaria, and rash. No previously unrecognized adverse reactions with Dukoral have been identified. The cumulative review of hypersensitivity reactions, cardiac disorders, as well as neurological or psychiatric disorders did not reveal any signals.

No cases with fatal outcome have been reported from the market in the time period from 1991 to the end of April 2014.

7.4.2.6 Summary of Safety Data

Overall, data from large-scale pre-licensure trials as well as over 20 years of post-marketing experience testify to the efficacy and safety of this vaccine. The adverse reactions observed with Dukoral are largely considered minor, self-limited reactions, such as gastrointestinal disorders and pyrexia. Almost 50% of adverse drug reactions reported with Dukoral are gastrointestinal disorders.

Data on overdose are limited. A total of 55 cases of overdose with Dukoral were reported since first approval in 1991, covering intake of two doses of Dukoral at the same time to intake of two doses within 6 days. Of these 55 cases, 2 cases were associated with non-serious adverse drug reactions: one report described nausea and three to four bowel movements after intake of two doses of Dukoral within 24 hours, while the second case reported nausea after intake of the second dose of Dukoral on Day 6. These adverse reactions reported after overdose is consistent with those seen after the recommended

dosing. Hence, the MAH (Marketing Authorization Holder) concludes that no safety issues are expected related to the different formulation of the proposed candidate vaccine VLA1701 compared to Dukoral.

Overall, no new significant safety related information has become available for Dukoral.

No significant change in the characteristics of listed reactions, e.g. severity, outcome, target population, has been noted during recent post-marketing experience.

In conclusion, Dukoral continues to have a favorable benefit-risk profile.

7.5 Challenge Strain

The LSN03-016011/A strain was isolated in 2003 from a 29 year-old U.S. female military dependent stationed in Turkey with watery non-inflammatory diarrhea and abdominal cramps. Co-infection with standard bacterial enteropathogens was ruled out by stool culture. The specimen was streaked onto MacConkey (MAC) agar plates, and 5 distinct E. coli-like lactose-fermenting colonies were inoculated into Tryptone Soy Broth (TSB). The TSB culture was archived with 15% glycerol and sent to Naval Medical Research Unit-three (NAMRU-3) in Cairo, Egypt for storage at -70°C. The Lsn03-016011/a isolate (one of the 5 isolated colonies selected from MAC plate) was confirmed as LT+ and CS17+ at NAMRU-3. The isolate was minimally passaged to generate a cGMP Master Cell Bank (MCB) as follows: the original archived LSN03-016011 isolate was streaked onto a MAC agar plate, and three isolated colonies (designated A to C) were streaked onto an agar slant and transferred to the Walter Reed Army Institute of Research/Naval Medical Research Center (WRAIR/NMRC). WRAIR/NMRC personnel confirmed LSN03-016011/A (i.e., colony A from the MAC plate) was CS17+ LT+ ST-. The isolate was expanded from the agar slant into luria broth (LB) and stored frozen with glycerol to generate a research seed master cell bank (RS MCB). This RS MCB was serotyped as 08:H- by E. coli serotype reference laboratory A. Cravioto, Universidad Nacional Autonomia de Mexico. This RS MCB was streaked onto MAC, serially expanded in LB to give an intermediate process development cell bank, a final process development cell bank, and finally the cGMP MCB. These steps were designed to ensure the cGMP MCB was E. coli only (purity). The cGMP MCB is E. coli only, and the toxin type and colonization factor type are consistent with those of the original isolate. The strain is sensitive to ampicillin, ciprofloxacin and sulfamethoxazole-trimethoprim.

7.5.1 Prior experience with the LSN03-016011 strain

LSN03-016011/A was previously used in a challenge study at Johns Hopkins (McKenzie JID 2011). An initial group of 5 subjects were challenged with 7 x 10^8 cfu. Three of the 5 (60%) developed diarrhea: 1 mild and 2 severe. Two subjects had vomiting, and 4 had abdominal cramps. When subsequently, 8 subjects were challenged with 6 x 10^9 cfu, 7 of them (88%) developed diarrhea: 4 moderate and 3 severe, but all 8 of the subjects were symptomatic. 75% had cramps and 37% had vomiting. It has subsequently been used in an additional group of 12 subjects during a passive protection study (Porter, JVAC2011, PLOSONE2016, unpublished data). The dose given to those subjects was 5 x 10^9 cfu, with NaHCO3 buffer for 2 days before and 5 days after challenge. In that group 50% developed diarrhea.

There were no unexpected adverse events upon challenge with the LSN03-016011 strain.

7.5.2 Challenge strain dose and justification

The challenge strain (E. COLI STRAIN LSN03-016011/A (LT+, ST-, CS17)) dose which will be used in the study will contains of fresh plate grown organisms will be used for challenge inocula, a standard approach for ETEC challenge studies. Approximately 48 hours before challenge, a vial of the cGMP MCB will be thawed and streaked onto colonization factor antigen agar (CFA with bile salts [CFA+BS] agar) and MAC agar (to document purity of the cGMP MCB). After 22 - 24 hours of incubation at 35 - 37o C, 10 colonies that agglutinate in CS17 antiserum will be used to prepare a suspension in sterile saline (0.85%). This suspension will be used to heavily inoculate 6 CFA+BS agar plates for incubation at 35 - 37o C. CFA + BS agar plates will be harvested in sterile saline after 18 - 20 hours and the resulting bacterial suspension further diluted in saline for optical density determination at 600 nm. The optical density of the suspension will be adjusted to the appropriate concentration of bacterial cells depending on study group. The number of cfu in the inoculum will be determined by titrating and plating on Luria agar plates before and after administration to subjects. The final inoculum will be examined by Gram stain for purity and for CS17 expression by agglutination with CS17 antiserum.

A sodium bicarbonate (USP-grade) solution of 2g/150 ml water will be prepared. Each subject will drink 120 ml of this buffer one minute prior to ingesting the challenge inoculum. Subjects will drink the challenge inoculum (approximately $5x10^9$ colony forming units) dissolved in the remaining 30 ml of buffer.

7.6 Study Rationale

The LSN03-016011/A strain challenge has been administered to a total of 48 human subjects, including only 25 naïve subjects, at doses ranging from 5×10^8 to 5×10^9 cfu. There is only limited data available from subjects in a placebo group who received the intended dose of $5x10^9$ cfu in a study setup suited for testing vaccine candidates. The primary objective of this study is therefore to confirm the characteristics of the challenge strain and secondary as well as exploratory endpoints will investigate safety and immunogenicity of VLA1701.

7.7 Evaluation of Anticipated Risks and Benefits of the Investigational Product(s) to Human Subjects

Naturally acquired and experimentally induced illness caused by ETEC ranges from mild-to-severe watery diarrhea. Nausea, vomiting, abdominal cramping, headache, abdominal gurgling or gas, anorexia, fever, muscle and/or joint aches, and malaise, may occur. For most adults the illness is not life threatening but often leads to mild to moderate dehydration and significant inconvenience associated with loss of sleep and activity. Study facilities will have personnel and resources capable to manage diarrheal illness and potential complications. Side effects to the antibiotic (ciprofloxacin) used to treat the ETEC infection are possible.

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 [Thabane M, et al., 2009]. PI-IBS, a functional bowel disorder characterized by unexplained abdominal discomfort or pain associated with changes in normal bowel patterns, has been described in a recent systematic review to occur 6-7 times more frequently after an acute enteric infection compared to similar matched controls without such a history [Thabane M, et al., 2009].

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 a 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.

Good clinical practices are performed during blood draws, which minimizes the risk to the subject. Hand-washing and sanitary disposal of feces are the main elements of personal hygiene and will minimize the spread by person-to-person infection; hand washing will be emphasized to the subjects and subjects will be instructed not to share food or beverages. Subjects and staff will be trained in proper techniques of hand washing. Subjects will be instructed as to the importance of completing the 3-day course of antibiotics and this instruction will be documented. Staff will be trained in the proper disposal of feces including pretreatment with bleach. Risk of secondary transmission is highly unlikely due to antibiotic treatment and because subjects are required to submit two confirmed, consecutive negative stool samples prior to discharge.

There is a minimal risk of pain, hematoma or infection at the site of venipuncture. Some subjects may feel lightheaded or faint during a blood draw. The maximum amount of blood drawn from a subject in total, and daily, will fall within applicable regulations.

Intravenous catheters placed for fluid replacement may also have the risk of pain, hematoma or infection at the site of the puncture.

There may be physical, psychological and social risks if subjects test positive for hepatitis B, hepatitis C and/or HIV. Positive tests must be reported to local health agencies. Subjects testing positive will be counseled and referred for treatment.

Use of the challenge bacteria carries with it a potential risk to the community of transmission from study subjects to others. To mitigate this risk, subjects will be confined to an isolation facility during the time that they may be infectious to others. Confinement to the facility may be stressful as well as boring. Subjects will be screened by the study staff for compatibility with the requirements of the study and given time to acclimatize to the study environment before vaccine is administered. Television, internet connections, and a variety of entertainment options will be available to study subjects during their inpatient stay.

Medical records associated with this protocol are subject to provisions of the Privacy Act of 1974, 5 U.S.C., Section 552A, and AR 340-21. All data and medical information obtained about subjects will be considered privileged and held in confidence to the extent possible. Subjects will not be identified by name in any published report/presentation of the results.

Positive HIV, Hepatitis B, or Hepatitis C tests will be reported to the appropriate health agencies. In addition, representatives of the Sponsor, JHSPH IRB, FDA or other regulatory agencies may inspect the records of this research as part of their responsibility to oversee research and ensure protection of subjects. Study results and data may be published in scientific/medical journals; the identity of individual subjects will not be disclosed.

7.7.1 Possible Benefits for Subject and for the Society

There is no benefit that can be guaranteed to subjects for participating in this research study. However, there is potential social benefit should this vaccine be able to prevent ETEC infection.

7.7.2 Possible Risks / Inconveniences for the Subject

The exact formulation of VLA1701 has not been evaluated in humans before. However, the individual components are contained in Dukoral in its licensed formulation and the same concentrations of overall bacterial count of whole cell killed vibrios (see section 7.4 and section 7.4.1), rCTB, LPS and formalin are present in VLA1701. Referencing from the established safety profile of Dukoral possible adverse events are likely to be minor, self-limited reactions, such as gastrointestinal disorders and pyrexia. Almost 50% of adverse drug reactions reported with Dukoral are gastrointestinal disorders.

Following challenge subjects will be questioned and examined daily for evidence of infection and diarrhea complications. Vital signs will be recorded at least three times per day. Based on prior studies, infected subjects tend to develop illness with incubation periods of approximately 1-3 days. Therapeutic benefit seems to be optimal if treatment is given within the first three days of symptom onset. 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. The plan will be to treat all subjects no later than day 5 post-dosing.

Aggressive fluid management will be undertaken to ensure the most common complication, dehydration, does not occur. The procedures to institute early oral and/or intravenous rehydration therapy are detailed above. In addition to rehydration therapy, prospectively defined criteria and procedures to institute early antibiotic therapy are also fully described above. In order to ensure clinical resolution and limit the potential for secondary spread upon discharge, predefined discharge criteria have been established. Subjects will be discharged from the inpatient phase of the study when clinical symptoms are resolved or resolving, they have received at least two doses of antibiotics AND two consecutive stool cultures are negative for ETEC.

Systemic or severe gastrointestinal complications rarely occur with ETEC infection. The following clinical findings necessitate immediate consideration and management of complicated enteritis:

- Physical examination compatible with an acute abdomen
- Severe GI bleeding (any evidence of GI blood loss other than hemoccult positivity only, with evidence of hemodynamic instability, decrease in hemoglobin, hypovolemia)
- Sepsis (high fever: temp. >102°F (39°C), rigors, hemodynamic instability).

Any of these findings require prompt clinical management and discussion with the independent Research Monitor.

The ETEC strain has the potential for risk to both the environment and to the research personnel; however, the risk to the environment in regards to potential transmission outside of the JHU CIR facility is low. There is a minimal risk of acquiring ETEC infection associated with subject inoculum administration, patient care activities on the ward, or processing ETEC-infected stool. The risk to the environment will be reduced by ensuring that all human waste products from inpatients are disinfected with bleach prior to disposal, ensuring all subjects comply with discharge criteria (two consecutive negative stool cultures for ETEC), emphasizing importance of handwashing for subjects and staff, ensuring proper disposal/cleaning of linen, and cohorting subjects in the JHU CIR while shedding ETEC. Additionally, subjects will not be discharged until they are no longer shedding the challenge strain as per procedures outlined in the protocol.

To mitigate the risk of PI-IBS, subjects with prior history of abnormal bowel patterns who might be at higher risk of this post-infectious sequelae are excluded and predefined criteria to assure early treatment as appropriate also may further reduce risk of post-infectious sequelae and is likely to reduce the risk associated with PI-IBS given the positive association between diarrheal illness duration and PI-IBS risk [Thabane M, et al., 2009].

8. STUDY PURPOSE AND OBJECTIVES

8.1 Study Purpose

8.1.1 Primary Objective

- The primary endpoint of this study is the number of subjects with moderate to severe diarrhea after challenge with ETEC strain LSN03-016011/ A.

8.1.2 Secondary Objectives

- To assess the scale of disease severity following Human Challenge with ETEC strain LSN03-016011/ A as described by Porter, et al; An Evidenced-Based Scale of Disease Severity following Human Challenge with Enterotoxigenic Escherichia coli; Plos one, 2016.
- To assess the safety of VLA1701 in a healthy adult population age 18 to <50 years.

8.2 Overall Study Design

This is a single-center, double-blind, placebo-controlled, Phase II vaccination and challenge study designed to confirm a human challenge model with E. coli strain LSN03-016011/A (LT+, ST-, CS17), as well as collect expanded safety and immunogenicity data.

The study will be carried out in two phases:

Vaccination phase: up to 34 subjects will be randomized 1:1 to receive 2 doses of either VLA1701 or placebo orally. The doses will be given 7 days apart and subjects will be followed as an outpatient for safety.

Challenge Phase: 30 Subjects, out of the 34 subjects, will be selected for challenge. After admission to the inpatient unit, on the following morning, and after approximately 90 minutes of fasting, 30 subjects will ingest 120 ml of USP sodium bicarbonate solution (13.35 g/liter) followed in 1 minute by another 30-ml bicarbonate solution containing the challenge strain. Subjects continue to fast for 90 min following challenge.

After challenge, subjects will be monitored for diarrhea and other signs/symptoms of enteric illness by daily medical checks, vital signs, grading and weighing of all stools.

Five days after challenge, or sooner if subjects meet early treatment criteria, subjects will be treated with antibiotics. Subjects will be discharged after at least 2 doses of antibiotic treatment, clinical symptoms are resolved or resolving and the subject has produced two stool samples that were negative for LSN03-016011/A by microbiological culture. All subjects will be followed up with an in-person visit at day 44 after vaccination (i.e., 28 days after challenge); a telephone call to check for any serious medical conditions, new onset of chronic illnesses and functional bowel disorders will be scheduled approximately 6 months after their first vaccination.

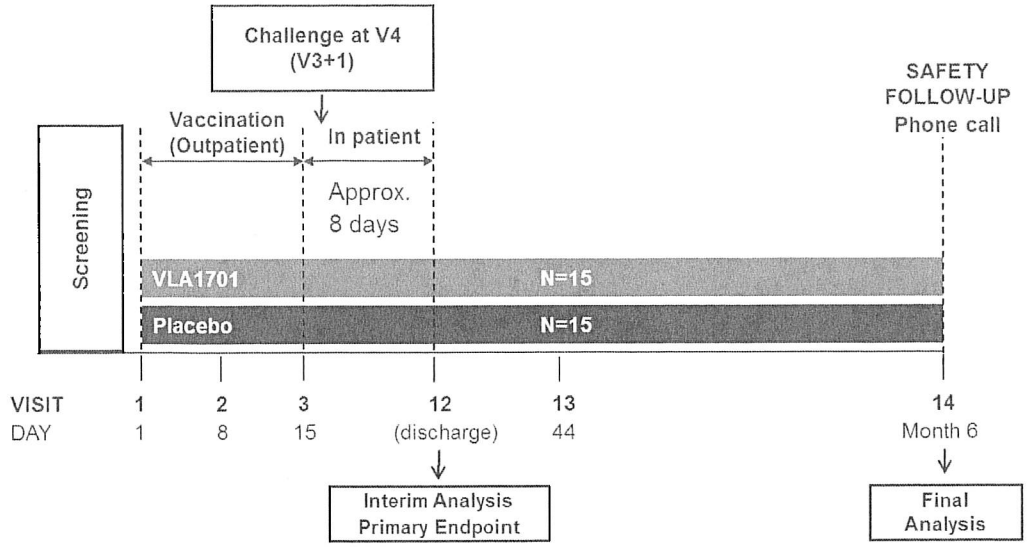

Figure 2: study design overview

8.3 Recruitment

The CIR at JHBSPH recruits subjects from the greater Baltimore, Washington DC and Philadelphia regions. Frequently, subjects are recruited from as far as the New York City metropolitan area.

Advertising is conducted via newspaper, radio, community ads, web-based and social media. Study fliers may be posted on the JHU campus and community bulletin boards. Additionally, subjects in previous studies that have expressed interest in participating in future trials will be contacted about the proposed study. All study specific-related advertisements will be reviewed and approved by the JHSPH IRB, NMRC IRB and HRPO-ORP. Subjects responding to the advertisements by a phone call to the center will be pre-screened for eligibility based on a standard pre-screening questionnaire administered by the CIR recruiter. Some elements of the inclusion/exclusion criteria will be discussed with the subject at that time and a preliminary determination will be made regarding the individual's eligibility for study participation.

8.4 Screening – according to protocol JH200

The CIR may use a screening protocol approved by the Johns Hopkins School of Public Health (JHSPH) Institutional Review Board (IRB) in recruiting subjects for this study. The screening protocol is entitled: "Screening of adult subjects for eligibility to participate in clinical studies evaluating investigational vaccines, antimicrobial agents, or disease prevention measures or the pathogenesis of infectious agents" JHSPH IRB 200, JHSPH IRB H.22.04.02.19.A2. Subjects will be made aware that the screening process may take several visits to complete. The screening protocol allows for the collection of a medical history, medical 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 with differential), serum chemistries, Hepatitis B antigen, Hepatitis C antibody, HIV-1 antibody, IgA levels, ABO and RH blood typing,

serum HCG, urine toxicology (drug screen), and immunological assessments. (Confirmatory testing will be performed on subjects who test positive for Hepatitis B, Hepatitis C, or HIV-1 antigens.) Subjects who have mild (grade 1) abnormalities may be included if the principal investigator determines that their participation will not present undue risk to the subject. Subjects with clinical laboratory abnormalities of greater than mild severity will not participate in this clinical trial.

8.5 Randomization

Subjects will be randomized in a 1:1 ratio to either receive VLA1701 or placebo. An unblinded statistician at Assign Data Management and Biostatistics will prepare a randomization list. Actual randomization of eligible subjects will be performed by site staff using the enrolment module of Advantage eClinical.

8.6 Blinding

To maintain the double-blind aspect of the study, the IMP will be prepared by unblinded pharmacy staff that is not participating in study drug administration or interacting with study subjects in any other way. The study staff, including the laboratory analysts, participating in other aspects of the study will be blinded to the treatment.

In compliance with applicable regulations, in the event of a SUSAR related to the blinded treatment, the subject's treatment code will usually be unblinded before reporting to the health authorities, ethic committees and investigators.

8.6.1 Emergency unblinding by the investigator

Emergency unblinding can be done by the PI contacting the unblinded pharmacist after consulting with the independent Research Monitor and, if possible, with the Sponsor. Drug identification information is to be unblinded only in the event that this is required for subject safety.

In the event that unblinding has occurred, the circumstances around the unblinding (e.g., date, time and reason) must be documented and the unblinded Clinical research Associate (CRA) notified as soon as possible. Only the Principal Investigator or delegate and the respective subject's code will be unblinded. Sponsor personnel directly associated with the conduct of the study will not be unblinded.

If the study site needs to unblind a subject, the sponsor will, if possible, be contacted prior to breaking the blinding. In all cases, the Medical Monitor must be notified within 24 hours after emergency unblinding.

Details regarding (un)blinding procedures will be described in a Study Specific Procedure.

8.7 Study Duration

The overall duration of the study is estimated to be about 7-8 months from study initiation (first subject enrolled to study completion (last subject last contact)). A subject will be considered enrolled in the study if he/she receives at least 1 dose of the vaccine. They will be considered completing the study if he/she finishes Visit 13.

The individual subject participation is approximately 6 months from enrollment to study completion unless prematurely discontinued.

8.8 Study Endpoints

8.8.1 Primary Endpoint

- Percentage of subjects with severe to moderate diarrhea within 120 hours of challenge with ETEC strain LSN03-016011/A.

The Definition of diarrhea is as follows (see also section 13.2.1.4):

During the inpatient period, each stool passed is collected, weighed, and graded as follows: grade 1, firm formed; grade 2, soft formed; grade 3, viscous opaque liquid or semiliquid which assumed the shape of the container; grade 4, watery opaque liquid; and grade 5, clear watery or mucoid liquid.

Stools defined as grade 3, 4, or 5 are considered to be loose and to potentially contribute to an episode of diarrhea:

- Severe diarrhea: at least six loose stools or > 800 g in 24 h;
- Moderate diarrhea: 4-5 loose stools or 401 to 800 g in 24 h;
- And mild diarrhea: 1-3 loose stool or ≤ 400 g in any 24-hour period.
- In calculating the total number and weight of diarrheal stools following challenge only stools which contributed to an episode of diarrhea according to these definitions will be included.
- An episode of diarrhea will be considered complete after 24 hours without a loose stool.

8.8.2 Secondary Endpoints

Severity of disease induced after challenge with ETEC strain LSN03-016011/ A

- Calculation of disease severity score after challenge with ETEC strain LSN03-016011/ A including "expected events" such as diarrhea (diarrhea score assigned by the highest score determined by either maximum 24 hour output volume or frequency), fever, nausea, bloating, vomiting, generalized myalgia, arthralgia, abdominal pain, abdominal cramping, malaise, headache, lightheadedness.

Safety of VLA 1701

- Percentage of subjects with solicited adverse events within 7 days after each vaccination.
- Percentage of subjects with any adverse events (AEs) observed up to Visit 4 (before Challenge) and during the entire study period (including clinically significant laboratory parameter changes).
- Percentage of subjects with serious adverse events (SAEs) observed up to Visit 4 (before Challenge) and during the entire study period.

- Percentage of subjects with any IMP-related AEs observed up to Visit 4 (before Challenge) and during the entire study period (including clinically significant laboratory parameter changes).
- Percentage of subjects with IMP-related SAEs observed up to Visit 4 (before Challenge) and during the entire study period.

8.8.2.1 Exploratory Endpoints

Disease induced after challenge with ETEC strain LSN03-016011/ A

- To quantify the number and quality of loose stools and grade the intensity of symptoms occurring after challenge.
- Number and percentage of subjects with diarrhea of any intensity.
- Number and percentage of subjects with diarrhea of any intensity, based on evaluation of volume only.
- Time to onset of diarrhea from the time of receiving the ETEC challenge (incubation period).
- Time, in hours, from the first diarrheal stool to the last diarrheal stool (duration of diarrhea).
- Mean total weight of grade 3-5 stools passed per subject.
- Mean number of grade 3-5 stools per subject.
- Total mass/volume of diarrheal stools.
- Maximum 24-hour stool output (volume and frequency).
- Number and percentage of subjects requiring early intervention with antibiotic therapy due to the severity of diarrhea.
- Number and percentage of subjects with fever, nausea, bloating, vomiting, myalgia, arthralgia, abdominal pain, abdominal cramping, malaise, headache, lightheadedness.
- Number of colony forming units (cfu) of the challenge strain per gram of stool on days 2 and 4 after challenge (V6 and V8).
- Number of subjects requiring IV fluids.
- Number and percentage of subjects developing moderate to severe diarrhea by ABO blood type.

Immunogenicity of VLA1701:

- Systemic immune responses after two doses of VLA1701 at 7 days post vaccination
- Mucosal immune responses after two doses of VLA1701 at 7 days post vaccination
- Systemic immune responses after challenge with ETEC strain LSN03-016011/A at 7 days and 1 month after challenge
- Mucosal immune responses up to 1 month after challenge with ETEC strain LSN03-016011/A at 7 days and 1 month after challenge

Blood will be collected per the Time and Events Schedule from subjects to assess for cholera and ETEC serum IgA and IgG responses.

The serum will be processed at the CIR laboratory and assayed for IgG and IgA antibody titers against LT using anti-ganglioside M1 (GM1)-enzyme-linked immunosorbent assay(ELISA), against CS17 using previously described methods. For all antigens, pre- and post-dosing serum samples from the same individual will be tested side by side. The antibody titer ascribed to each sample will represent the geometric mean of duplicate determinations. Reciprocal endpoint titers <50 will be assigned a value of 25 for computations. Seroconversion is defined as two-fold increase in endpoint titer between pre- and post-challenge specimens AND a post-challenge reciprocal titer > 100.

PBMCs will be assayed to determine antigen specific (CS17, LPS, and LT) ALS responses. PBMCs are incubated without stimulation and the supernatant is later assayed for antigen specific IgA antibodies by ELISA. A positive ALS response will require a four-fold rise in antibody titers between pre and post challenge samples.

Antibody in lymphocyte supernatant assay (ALS): Venous blood samples will be collected and peripheral blood mononuclear cells will be isolated and processed for ALS assay.

Fecal IgA: Fecal samples will be collected and processed. Total IgA and anti-LT/CT IgA responses in fecal samples will be measured before first vaccination, day before challenge, 7 and 28 days after challenge. Anti CS17 IgA will be measured on day before challenge and 7, 28 days after challenge. Antigen specific fecal IgA titers will be expressed as units per milligram of total IgA.

Vibriocidal Titers: Vibribiocidal assays will be done using serum before first vaccination, day before challenge, 7 and 28 days after challenge.

8.8.2.2 Microbiological

During the inpatient study (Visit 3 to Visit 12), stool samples (at least 1 per subject per day post-inoculation) or rectal swab, if necessary, will be screened for the presence of LSN03-016011/A. Samples will be collected, processed and shipped as per the SSP, for qualitative cultures. Up to 5 E. coli-like colonies from MacConkey selective media will be subcultured onto CFA without bile salts agar and then screened for agglutination with challenge strain-specific antiserum using a slide agglutination technique.

Additional culture-independent methods may be used to quantitate LSN03-016011/A shedding.

8.8.2.3 Outcome Evaluation

In an effort to obtain an unbiased determination of the efficacy outcomes, an independent outcome adjudication committee, the members of which will be blinded as to the treatment regimens of the subjects, will evaluate challenge outcome data after completion of the inpatient phase of the study (see section 17.8).

9. SUBJECT SELECTION, WITHDRAWAL AND DISCONTINUATION

9.1 Study specific screening/ eligibility

At the study specific screening visit (Visit 0) and at Visit 1 (Day 1) subjects' inclusion and exclusion criteria will be checked (including laboratory results obtained from screening analysis) and eligibility of the subjects to participate in the trial confirmed. A review of pertinent inclusion and exclusion criteria will occur before the second vaccination and before challenge to ensure that subjects remain eligible.

Women of child bearing potential have to agree to use effective contraception for the duration of the study and must have a negative urine pregnancy test prior to each vaccination and prior to challenge.

Approximately 34 adults of both genders, who satisfy the inclusion and exclusion criteria listed below, will be invited to participate in the study.

9.2 Inclusion Criteria

Subjects who meet **ALL** of the following criteria are eligible for this study:

1. Healthy male and non-pregnant female subjects age 18 to <50 years; health status is assessed by investigator at time of screening based on medical history, physical examination, and laboratory parameters.
2. BMI of 19.0 to 35.0 kg/m2
3. Willingness to participate after informed consent obtained from the subject prior to any study related procedures.
4. Completion of a training session and demonstration of comprehension of the protocol procedures and knowledge of ETEC-associated illness by passing a written examination.
5. If subject is of childbearing potential:
    a) Negative pregnancy test at screening (Visit 0) with understanding to not become pregnant within 28 days after challenge.
    b) Subject has practiced an effective method of contraception (see below) during the 30 days before screening (Visit 0);
    c) Subject agrees to employ adequate birth control measures for the duration of the study. This includes one of the following measures:
        - Hormonal contraceptives (e.g. implants, birth control pills, patches);
        - Intrauterine device;
        - Barrier type of birth control measure (e.g. condoms, diaphragms, cervical caps);
        - Vasectomy in the male sex partner ≥3 months prior to first vaccination.

9.3 Exclusion Criteria

Subjects who meet **ANY** of the following criteria are **NOT** eligible for this study:

1. Participated in research involving investigational product within 30 days before planned date of first vaccination or planned use through Day 44;
2. Any prior exposure to ETEC (including LSN03-016011/A) or cholera occupationally or received LT (Or any mutant forms of LT (eg, LTR192G, LTR192GL211A), ETEC, or cholera vaccine);
3. Subjects with known abnormal stooling patterns (fewer than 3 per week or more than 3 per day);
4. Known allergies to any component of the vaccine;
5. Subjects with known allergies to more than 1 planned antibiotics: Ciprofloxacin, Amoxicillin, trimethoprim-sulfamethoxazole;
6. History of diarrhea while traveling in a developing country within the last 3 years;
7. Subjects whose occupation involves handling of ETEC or cholera bacteria;
8. Women who are pregnant or breastfeeding;
9. Significant medical conditions including chronic, immunosuppressive, malignant, or gastrointestinal diseases (e.g. History of Irritable Bowel Syndrome (as defined by the Rome III criteria or medical diagnosis) or gastric ulcer disease) or enteric, pulmonary, cardiac, liver or renal disease. Some medical conditions which are adequately treated and stable may be acceptable in the study (e.g. hypertension);
10. Significant abnormalities in screening lab hematology or serum chemistries, as determined by PI or PI in consultation with the independent Research Monitor and sponsor;
11. Use of any medication known to affect the immune system (e.g. systemic corticosteroids) within 30 days of vaccination or planned use during active study period (excluding inhaled steroids);.
12. Evidence of confirmed infection with HIV, Hepatitis B or Hepatitis C;
13. Subjects with IgA deficiency (serum IgA < 7 mg/dl or limit of detection of assay);
14. Regular use of antacids, antidiarrheal, loperamide, bismuth subsalicylate, diphenoxylate or similar medication less than 2 weeks prior to enrolling in the study and through the inpatient portion of the study.
15. Known or suspected alcohol abuse or illicit drug use within the last year, positive urine toxicology for opioids, benzodiazepines or amphetamines.
16. Persons who are committed to an institution (by virtue of an order issued either by the judicial or the administrative authorities).
17. Persons who are in a dependent relationship with the sponsor, an investigator or other study team members, or the study center. Dependent relationships include close relatives and household members (i.e. children, partner/spouse, siblings, and parents) as well as employees of the investigator or study center personnel.

18. 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.

9.4 Selection of randomized and vaccinated subjects for challenge

Subjects meeting the following criteria will not be considered eligible for challenge and should follow the schedule for outpatient visits only (i.e. Visit 12 and 13).

- Subjects who did not receive both doses of IMP
- Use of antibiotics with a known or theoretical ability to modulate ETEC risk between the second vaccination and challenge.
- Subjects with ongoing adverse events that may preclude assessment of primary or secondary outcomes during the inpatient phase of the study.
- Subjects who, in the opinion of the clinical investigator, would not be suitable for an inpatient setting.

Further, since there is a maximum capacity of 30 subjects for the inpatient phase of the study, it may be necessary to exclude subjects considered to be eligible before challenge. After performing eligibility assessments on the day of admission (Visit 3), the study site will provide a list of all subjects that are considered eligible for challenging to the unblinded study statistician who will then select 30 subjects together with two backup subjects (if available). The selection list will be provided back to the study site. All subjects on the list will be admitted for the overnight stay between Visit 3 and Visit 4. Subjects dropping out before Visit 4 will be replaced with backup subjects.

The following rules will be applied for subject selection for challenge (ordered by priority):

1. A maximum of 30 subjects can be challenged.
2. There are three 4-bed rooms and three 6-bed rooms. Men and women must not share a room.
3. The vaccine-placebo ratio should be as close to 1:1 as possible.
4. The gender distribution should be as close to 1:1 as possible.
5. Up to two subjects are selected as backup subjects, if possible one female and one male
6. If two subjects of same sex and treatment group are eligible, the subject with the lower randomization number has higher priority for selection.

Details are summarized in a separate document ("Randomization and challenge subject selection", final 2.0, 05-Apr-2018).

9.5 Pregnancy Testing and Birth Control

A woman is considered of childbearing potential if fertile, following menarche and until becoming post-menopausal unless permanently sterile.

Women of childbearing potential are required to practice an acceptable method of birth control throughout the active (through Visit 13) study period. An effective method of birth control is defined as those, which result in a low failure rate (i.e. less than 1% per year) when used consistently and correctly.

This includes one of the following measures:

- Hormonal contraceptives (e.g. implants, birth control pills, patches);
- Intrauterine device;
- Barrier type of birth control measure (e.g. condoms, diaphragms, cervical caps);
- Vasectomy in the male sex partner ≥ 3 months prior to first vaccination;
- Not to be of reproductive potential, such as having undergone hysterectomy, bilateral oophorectomy, or tubal ligation.

A woman is considered of non-childbearing potential, if she is:

- Surgically sterilized for ≥ 3 months prior to Visit 1 (permanent sterilization methods include hysterectomy, bilateral salpingectomy or bilateral oophorectomy, or transcervical sterilization (Essure and Adiana procedures);
- Postmenopausal for ≥ 1 year prior to study start as confirmed by a gynecologist.

Women must not become pregnant during the active study period, up to Visit 13. If a subject becomes pregnant during the active study, she must immediately inform the Investigator. Study vaccination and/or challenge should be suspended, if applicable, and the subject is asked to complete all remaining outpatient follow-up activities according to study schedule for safety. Pregnancies reported to the investigator after Visit 13 are not planned to be followed-up.

9.6 Subject Withdrawal or Discontinuation

Any subject has the right to withdraw from the study at any time for any reason, without the need to justify. Counseling about the subject's health will be provided if he/she decides to discontinue participation in the study. Medical advice regarding what is in the best interest of the subject will be provided. The Investigator and Sponsor also have the right to prematurely terminate a subject's further participation in the study, e. g. in the case of non-compliance or if – in the judgment of the Investigator and/or Sponsor – continued participation would pose an unacceptable risk for the subject. Subjects will also be withdrawn from further vaccination or discontinued from further study participation for the following reasons:

- The subject becomes pregnant prior to completion of Visit 13. Study vaccine exposure will be discontinued, and the subject will not be challenged. Attempts will be made to follow her through completion of the pregnancy. The Investigator will record a narrative description of the course of the pregnancy and its outcome. For further information on pregnancy reporting procedures see Section 13.8.
- The subject experiences a severe systemic allergic reaction, e.g. generalized urticaria within 72 hours after vaccine administration, or anaphylaxis within 24 hours following vaccine administration with no likelier alternative cause than the study vaccine;

The primary reason for withdrawal / discontinuation of a subject from treatment and/or from the study should be documented in the electronic Case Report Form (eCRF) (e.g. withdrawal of consent, Investigator/Sponsor recommended withdrawal, lost to follow up, death).

The primary reasons for discontinuation will be reported on the Discontinuation eCRF, including:

- Withdrawal due to AE;
- Lost to follow-up (defined as 3 documented unsuccessful attempts to contact the subject[i]);
- Investigator decision (e.g. pregnancy, progressive disease, non-compliance with protocol);
- Study terminated by Sponsor;
- Death;
- or other (reason to be specified by the Investigator, e.g. technical problems).

Regardless of the reason, all data available for the subject up to the time of completion/discontinuation should be recorded on the appropriate CRF.

Subjects withdrawn from further vaccination should perform their remaining regular outpatient study visits as scheduled if there are no other reasons for premature withdrawal from the study.

Up to 4 alternates may be vaccinated but not challenged. For those subjects:

- They should come in for a day 7 and 28 follow up out-patient visit after their last vaccination.

Data collected on withdrawn subjects will be used in the analysis and included in the clinical study report.

A subject may be withdrawn for an adverse event (AE) or serious adverse event (SAE) resulting in a safety concern, or for noncompliance with protocol requirements. When a subject withdraws due to an AE or is withdrawn by the PI due to an AE, the sponsor must be notified within 24 hours. Investigators must follow specific policy at each institution regarding the timely reporting of AEs and SAEs to the local IRB. In all cases, the PI will make a reasonable effort to complete study termination procedures.

Subjects who do not complete the entire study due to withdrawal or discontinuation for any reason will not be replaced.

9.7 Follow-up for withdrawn subjects

If possible, attempts will be made to follow-up with the subjects for safety at least 28 days after receipt of vaccination and challenge inoculum. Immunogenicity assessments will be continued for all subjects presuming no undue risk to the subjects related to specimen collection. If a subject meets withdrawal conditions for a concomitant medication violation or noncompliance, this should be clearly documented. Subjects who receive challenge and subsequently withdraw from the study will receive antibiotics for outpatient treatment and will be educated on the importance of complying with treatment.

[i] **Note** that a subject who is lost to follow-up but later returns to the study site for a follow-up can still complete the study provided he/she has received all vaccinations according to the protocol.

10. INVESTIGATIONAL MEDICINAL PRODUCT

10.1 Description of VLA1701

The investigational medicinal product VLA1701 used for this pilot human challenge trial VLA1701-201 is an oral inactivated vaccine containing inactivated V.cholerae bacteria as well as rCTB:

+ A total of $1.25x10^{11}$ vibrios of the following strains:

- Vibrio cholerae O1 Inaba EL Tor strain (formalin inactivated) $6.25x\ 10^{10}$ vibrios
- Vibrio cholerae O1 Ogawa classical strain (heat inactivated) $6.25x\ 10^{10}$ vibrios

+ Recombinant cholera toxin B subunit (rCTB) 1 mg

VLA1701 contains an increased number of V. cholerae O1 bacteria of biotype El Tor since this is the causative organism of the seventh cholera pandemic that started in 1961. During the sixth pandemic all isolates were of the classical biotype. Both Ogawa and Inaba strains are included in line with the requirements for parenteral cholera vaccines as set forth in e.g. 21CFR 620.30 and Eur. Pharm. 1997:0154. The El Tor strain is formalin inactivated in order to protect heat sensitive antigens.

Clinically Relevant Nonmedicinal Ingredients: Sodium Hydrogen Carbonate (5.6 g sodium hydrogen carbonate, saccharin sodium).

For more details, please refer to the related current version of the IB.

10.1.1 IMP effervescent granules

The effervescent granules are a sachet of sodium hydrogen carbonate of 5.6 g in total. The granules are packaged in sachets made of white transofoil EL (laminate of polyester/ polyethylene/aluminium foil/polyethylene/-LD-polyethylen). Recipharm AB is responsible for manufacture, testing and release of effervescent granules.

10.1.2 Administration of VLA1701 and effervescent granules

The vaccine is administered orally in 2 doses at least 1 week apart. Subjects have to fast one hour before and 1 hour after each vaccination.

The vaccine is provided as 2 components:

a. active substance in a glass vial and

b. effervescent granules contain sodium hydrogen carbonate buffer in a sachet.

The effervescent granules are dissolved in an opaque cup of bottled water before adding the component from the glass vial. The vaccine is to be consumed in the presence of the buffer in order to protect the antigen from the acidic components in stomach.

10.2 Description of Placebo

The buffer component of VLA1701 will be used as placebo in this study (see 10.1.1). The effervescent granules are dissolved in a cup of bottled water (approximately 150 ml). In order to keep the blind towards study staff (other than the unblinded pharmacist) and the subjects,

an in-transparent cup will be used. In addition, subjects have to fast one hour before and 1 hour after each vaccination in line with the requirements for VLA1701.

10.3 Description and Administration of the Challenge Strain

Fresh plate grown organisms will be used for challenge inocula, a standard approach for ETEC challenge studies. Approximately 48 hours before challenge, a vial of the cGMP MCB will be thawed and streaked onto colonization factor antigen agar (CFA with bile salts [CFA+BS] agar) and MAC agar (to document purity of the cGMP MCB). After 22 - 24 hours of incubation at 35 - 37°C, 10 colonies will be used to prepare a suspension in sterile saline (0.85%). This suspension will be used to heavily inoculate 6 CFA+BS agar plates for incubation at 35 - 37°C. For purity checking 3-5 colonies from the plate will be checked using slide agglutination. Colonies will also be checked with Gram stain and streaking on MacConkey. CFA + BS agar plates will be harvested in sterile saline after 18 - 20 hours and the resulting bacterial suspension further diluted in saline for optical density determination at 600 nm. The optical density of the suspension will be adjusted to the appropriate concentration of bacterial cells depending on study group. The number of CFU in the inoculum will be determined by titrating and plating on Luria agar plates before and after administration to subjects. The final inoculum will be examined by Gram stain for purity and for CS17 expression by agglutination with CS17 antiserum.

A sodium bicarbonate (USP-grade) solution of 2 g/150 ml water will be prepared. Each subject will drink 120 ml of this buffer one minute prior to ingesting the challenge inoculum. Subjects will drink the challenge inoculum (approximately $5x10^9$ colony forming units) dissolved in the remaining 30 ml of buffer.

10.4 Packaging, Labeling, and Storage

10.4.1 Packaging

VLA1701 active substance will be provided in single-use vial. The effervescent granules are packaged in sachets made of white transofoil EL (laminate of polyester/polyethylene/aluminium foil/ polyethylene/-LD-polyethylen).

10.4.2 Labeling

The IMP will be labeled according to the applicable regulatory requirements for clinical trials.

10.4.3 Storage

The study site will maintain current drug shipment logs detailing the date received, drug identity code, date of manufacture or expiration date, amount received from and returned to the Sponsor.

All investigational products (vaccine, challenge strains) will be stored at the investigational site in accordance with GCP and GMP requirements and the instructions given by the clinical supplies department of the sponsor (or its affiliate/CRO), and will be inaccessible to unauthorized personnel. Special storage conditions and a complete record of batch numbers

and expiry dates can be found in the sponsor's study file; the site-relevant elements of this information will be available in the investigator site file.

On the day of receipt, the responsible site personnel will confirm receipt of investigational products in writing.

The IMP must be stored at 2-8°C in a room not accessible to unauthorized persons. The vaccine should be protected from direct light. Storage at higher temperatures should be avoided because of potential impairment to immunogenicity and tolerability. Temperature monitoring systems will be used.

10.4.4 Dispensing and Accountability of IMP

The personnel will use the investigational products only within the framework of this clinical study and in accordance with this protocol. Receipt, distribution, return and destruction (if any) of the investigational products must be properly documented according to the sponsor's agreed and specified procedures.

Written instructions on investigational products destruction will be made available to affected parties as applicable.

A current vaccine /challenge-dispensing log has to be maintained, detailing the dates and quantities of IMP administered to each subject. Records will be maintained that includes subject identification code (SIC), dispensation date, and amount dispensed. This documentation will be available to the designated unblinded CRA to verify drug accountability during the study and to perform overall drug accountability.

Any unused IMP and empty vials and challenge will be accounted for and returned to the Sponsor or destroyed as per Sponsor and SSP.

Further details regarding preparation of VLA1701 and placebo, dispensation and accountability are provided in the VLA1701-201 IMP manual and SSPs.

10.4.5 Challenge strain information

Strain LSN03-016011/A was isolated in 2003 from a U.S. female military dependent stationed in Turkey, who developed watery non-inflammatory diarrhea and abdominal cramps. Fecal specimens with a study number INC-099-MI-1190 D0 were streaked onto Maconkey (MAC) agar plates, and 5 distinct E. coli-like lactose-fermenting colony forming units were inoculated into Tryptone Soy Broth (TSB). Microbiologic culture of the fecal specimen for standard bacterial enteropathogens apart from E. coli was negative. The TSB cultures were archived with 15% glycerol and sent to the Naval Medical Research Unit-three (NAMRU-3) at Cairo, Egypt for storage at -70°C. LSN03-016011/A is one of those five TSB cultures. The strain was confirmed as LT+ and CS17+ at NAMRU-3.

The LSN03-016011/A cell banks from Research Seed Master Cell Bank (RS MCB) to cGMP MCB were found to be LT+ and ST- by extracting plasmid DNA and Southern blotting with probes specific for LT, and ST. Presence of the gene for the major subunit of CS17 was confirmed by DNA sequencing of plasmid DNA from the RS MCB and cGMP MCB. The expression of CS17 was confirmed in all cell banks by SDS-PAGE and Western blotting with CS17-specific anti-serum.

The cryopreserved frozen stock of LSN03-016011/A was expanded at NAMRU-3 onto a MAC plate. The plate was incubated for 24h at 37°C, and only lactose-fermenting colonies with E. coli-like appearance were visible. Three well-isolated lactose-fermenting colonies

were selected from each MAC plate (designated A, B or C), and suspended in 200µl sterile TSB. A loop-full of the homogeneous suspension was streaked onto the surface of a Tryptone Soy Agar (TSA) slant and incubated overnight at 37°C then transferred to Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD where they were expanded in Luria-Bertani (LB) broth to generate a RS MCB.

The RS MCB stock was transferred to Cambrex Bio Science at Baltimore, MD, for isolation and expansion of a pure E. coli culture. The RS MCB was passaged onto MAC, and streaked on TSA and Xylose Lysine Deoxycholate (XLD) to assess purity. Ten lactose-fermenting colony forming units identified as LSN03-016011/A clones A to J, were isolated from the MAC plate and expanded in 10ml LB to give 10 Intermediate Process Development (I PD) cell banks, vialed with 15% glycerol and stored at -70°C. One of the LSN03-016011/A clones (Clone A) was selected for further passage based on its favorable characteristics. The clone was identified as CS17+ LT+ E. coli, and purity was confirmed by plating on MAC, TSA, Cetrimide (Cet), Mannitol Salt Agar (MSA) and Sabouroud-Dextrose Agar ± Chloramphenicol (SDA±Cm) plates.

The I PD seed was expanded in 50ml LB Broth to generate a Final Process Development (Final PD) cell bank, and transferred to McKesson BioServices (now Fisher Bioservices) for storage at -70°C. In advance of cGMP, cell banking vials were transferred to the Pilot BioProduction Facility (PBF) at WRAIR, MD. At this facility the Final PD stocks were expanded in LB to generate a cGMP MCB (lot 1285). The cell banks at each stage were identified as E. coli by VITEK® analysis at the Walter Reed Army Medical Center (WRAMC), DC, and typed as CS17+ LT+ ST- by Southern blot analysis at the Naval Medical Research Center (NMRC). The purity was confirmed by plating on selective agar.

11. STUDY PROCEDURES

11.1 Informed Consent and Enrollment

Informed consent is an ongoing process. Potential subjects will receive a presentation about the study. Each prospective subject will be given the written, IRB-approved informed consent, allowed ample time to read the consent, ask questions about the study, have his/her questions answered, and given time to decide if he/she would like to participate in the study. 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. A final acceptable test score is 70% or more answered correctly. Subjects not meeting 70% will be given one additional opportunity to review the presentation, consent and retake the quiz. Subjects failing after 2 attempts are not eligible for study enrollment. No coercion or influence is allowed in obtaining subjects' consent. 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.

11.2 Screening (Day -60 to -1)

11.2.1 JH200 Screening

The following procedures will be completed at the JH200 screening visit. Subjects will be consented to a general screening protocol as per Section 8.4 above. During this screening visit the following procedures may be performed:

- Provide HIV pretest counseling including information about HIV testing, transmission and prevention, explanation of test results, post-test counseling, result reporting and that the testing is voluntary, but necessary for study participation.
- Elicit a complete medical history, including menstrual and contraceptive history and/or history of surgical sterility for female subjects.
- Pregnancy prevention counseling for females.
- Vital signs will be collected (heart rate, respiratory rate, blood pressure and temperature)

Additionally, the following activities may occur or be deferred to a subsequent screening visit depending on study requirements:

- A complete physical examination
- Obtain approximately 25 mL of blood for a CBC with differential, Basic Metabolic Panel (BMP), ALT, Hepatitis B virus, Hepatitis C virus, and HIV. Obtain urine sample for toxicology testing. Females may also have a urine or serum sample for β-HCG testing.

11.2.2 Study Specific Screening

A study-specific screening visit may be scheduled between -30 to -1 days before the first vaccination.

Basic subject information, plus the subject identification code (see Section 11.2.3) will be recorded on the Screening Log.

The following evaluations/procedures will be carried out:

- Subjects must fully understand the elements of the Informed Consent form, and sign and date the form prior to initiating protocol–specific procedures not covered in the JH200 screening protocol.
- Subjects must take and pass (with > 70% understanding) a comprehensive test. Study staff will review any questions that the subject may have and the subject will be able to retake the comprehension test once if they do not pass the first time.
- Review Inclusion/Exclusion Criteria

  The inclusion and exclusion criteria will be reviewed by the investigator or qualified designee to ensure that the subject qualifies for the study (see section 9.2 and 9.3 for a detailed list of the criteria).
- Review/Obtain demographic data (date of birth, height, weight, BMI, gender and race), Medical History and update address and contact information as needed.
  A complete medical history will be obtained or reviewed and updated by study staff; reviewed by the investigator or qualified designee with the subject and maintained in the subject' records.
- Clinical chemistries and hematology (see section 13.6), Hepatitis B virus, Hepatitis C virus, and HIV, serum IgA level, blood typing. (if not completed during JH200 screen).
- Obtain urine sample for toxicology testing, testing for the presence of amphetamine, barbiturates, opiates, phencyclidine, benzodiazepine, methadone and propoxyphene. Females may also have a urine or serum sample for β-HCG testing (if not completed during JH200 screen).
- Physical examination
- Vital Signs
- IBS survey (i.e. Rome III)
- Stool sample: fecal total IgA and Anti LT/CT/IgA and other exploratory assays (may be collected any time prior to first vaccination)

11.2.3 Subject Identification Code

At Visit 0, a subject identification code will be assigned to each subject, which will be different from the identification code according to the JH200 screening procedure.

11.3 Vaccination Phase

11.3.1 Visit 1 (Day 1): Enrollment, 1st Vaccination

During V1, the following steps will be performed:

- Re-confirmation of subject eligibility (review of inclusion/exclusion criteria)
- Recording of any concomitant medication/treatment and/or vaccination. Review any new or change in medical history

- Focused physical examination and vital signs
- Blood/serum: LT/CT IgA, IgG, ALS, vibriocidal titers
- Stool sample: fecal total IgA and Anti LT/CT/IgA and exploratory assays (If not performed at Visit 0)
- Urine pregnancy test for female subjects
- Randomization
- Vaccination (Oral)
- Subjects will fast for 60 minutes before and after vaccination
- Subjects will be monitored for 30 minutes after vaccination for vomiting or other immediate adverse reaction
- Vital signs will be rechecked after 30 minutes, memory card and thermometer provided to subject with oral and written directions. See section 13.2.1.6.1 and 13.4.

11.3.2 Visit 2 (Day 8): 2nd Vaccination

Subjects will return to the center for their second vaccination and the following steps will be performed:

- Pertinent inclusion/exclusion criteria will be reviewed to assure subjects remain eligible to continue in the study
- Memory cards from Vaccination #1 collected and reviewed
- Females will have urine pregnancy testing completed prior to vaccination and test must remain negative
- Subjects will undergo focused physical examination and vital sign assessment, review of any new or change in AEs and medications they are or have been taking
- Vaccination (oral)
- Subjects will fast for 60 minutes before and after vaccination
- Subjects will be monitored for 30 minutes after vaccination for vomiting or other immediate adverse reaction
- Vital signs will be rechecked after 30 minutes, memory card and thermometer provided to subject with oral and written directions

11.4 Challenge Phase

11.4.1 Visit 3 (Day 15-18): Admission

Before admission, a blinded list with all subjects' IDs to be included for the challenge (with vaccine and placebo recipients equally distributed) will be provided to predefined study team members.

On the day of admission,

- Pertinent inclusion/exclusion criteria will be reviewed to assure subjects remain eligible to continue in the study

In addition, subjects meeting the following criteria will not be admitted and should follow the schedule for outpatient visits only (i.e. Visit 12 and 13).

  - Subjects who did not receive both doses of IMP
  - Use antibiotics with a known or theoretical ability to modulate ETEC risk between the second vaccination and challenge.
  - Subjects with ongoing adverse events that may preclude assessment of primary or secondary outcomes during the inpatient phase of the study.
  - Subjects who, in the opinion of the clinical investigator, would not be suitable for an inpatient setting.

- Memory cards from Vaccination #2 collected and reviewed
- Clinical chemistry and hematology (see 13.6)
- Subjects will undergo vital signs assessment (heart rate, blood pressure, and temperature), review of medical history, physical examination (PE) on admission.
- An ECG will be performed to ensure that individuals who have cardiac contraindications are not given ciprofloxacin but alternative antibiotic treatment.
- Changes regarding AEs and concomitant medication will be assessed.
- A serum pregnancy test (β-hCG) will be obtained from women of childbearing potential. If the result of a serum pregnancy test is not available on day of challenge, a urine pregnancy test will be conducted.
- Serum samples will be collected for assessment of immune outcome (and exploratory) measures as described in schedule of events.
- Stool will be collected for bacteriology, immunology and exploratory assays as per schedule of events (Section 11.7). In the event a subject is unable to produce a stool at admission, stool may be collected anytime from admission to up to 4 hours after challenge at the latest. A subject's inability to produce a stool will not be exclusionary for challenge, but it will be considered a protocol deviation for this study. Once a subject is challenged, an inability to produce stool on a given day will not be considered a protocol deviation.

One to 2 alternates may be admitted overnight to replace anyone who may not continue to meet eligibility criteria in the next morning, and the remaining vaccines will be considered outpatient alternates. The alternate or a subject who does not continue to meet eligibility criteria will be discharged without challenge and follow the schedule for outpatient visits only (i.e. Visit 12 and 13).

11.4.2 Visit 4: Challenge Day

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

Prior to challenge subjects will have:

- Eligibility criteria re-confirmed
- Vital signs (VS) (blood pressure, heart rate, temperature)
- A focused physical exam and evaluation to ensure that there are no changes from admission and no exclusionary conditions have arisen.
- Changes regarding AEs and concomitant medication will be captured.
- Approximately 1 minute prior to challenge, subjects will drink 120 mL of bicarbonate buffer.
- For the challenge, subjects will drink a solution of virulent *E. coli* strain LSN03-016011/A (LT+, ST-, CS17) in 30 mL of bicarbonate buffer.
- Vital signs and ETEC disease-specific expected events (see section 12) will be assessed at least 30 minutes after challenge.
- Subjects will continue fasting for at least an additional 90 minutes post challenge, after that, no dietary restrictions will be placed on the subjects.
- Subjects will have two additional sets of VS this day.
- All stools will be collected for weighing and grading. Grade 3, 4, or 5 stools shed after challenge will be collected and may be sent for the lab for culture.

11.5 Post Challenge Phase

11.5.1 Visit 5-8 (Day 1-4 post challenge): Inpatient monitoring

- Subjects will remain at the inpatient facility under clinical observation.
- Vital Signs (blood pressure, heart rate, and temperature) will be assessed at least thrice daily: in the morning, in the afternoon and in the evening.
- Changes regarding AEs and concomitant medication will be captured.
- A clinician will conduct a daily medical interview and focused PE to assess health status, follow-up, monitor, and treat as indicated.
- All stools will be collected for weighing and grading. Starting the day after challenge, up to 3 stool samples will be collected daily for culture. If a subject is unable to provide a stool sample by 01:00 pm, he/she will be asked to obtain a rectal swab.
- Treatment for vomiting may be needed. Subjects who are vomiting may be given ondansetron for the management of vomiting
- Antibiotic treatment after challenge will be administered according to criteria for early antibiotic treatment (described below) or 5 days (about 120 hours) after challenge if subjects do not meet the criteria for early treatment. Subjects will be treated with an antibiotic [ciprofloxacin (500 mg by mouth twice daily for 3 days)], or a suitable alternative. Alternative treatments are trimethoprim 160 mg / sulfamethoxazole 800 mg by mouth twice daily for three days, or amoxicillin (500 mg by mouth 3 times daily for 3 days). If, because of illness, a subject is unable to take oral antibiotics, intravenous antibiotics may be given.

- Subjects may be discharged from the inpatient unit after receipt of at least two doses of antibiotic treatment, clinical symptoms are resolved or resolving and the subject has produced two consecutive stool samples negative for LSN03-016011/A by microbiological culture.

During the challenge phase of the trial, a subject will qualify for early treatment (< 120 hours [5 days] after challenge) with ciprofloxacin (or trimethoprim-sulfamethoxazole or amoxicillin) if any of the following criteria are met post-challenge:

- Severe diarrhea (based on volume, 800 g in 24 hours)
- Stool output consistent with moderate diarrhea for 48 hours
- Mild or moderate diarrhea and 2 or more of the following symptoms: severe abdominal pain, severe abdominal cramps, severe nausea, severe headache, severe myalgia, any fever (≥ 38.0°C), or any vomiting
- A study physician determines that early treatment is warranted for any reason.

Rehydration Procedures

Subjects passing grade 3-5 stools post-challenge will be offered oral fluids such as ORS or Gatorade to prevent dehydration, at the same volume as their stool output.

A subject may be administered IV fluids (clinician discretion) 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 BP < 90 mmHg and associated symptoms, or significant lightheadedness on standing, with a confirmed postural change in BP or pulse. Postural vital signs will be measured lying and 2 minutes after standing. A significant change is a decrease in systolic BP of > 20 mmHg, or diastolic BP of > 10 mmHG or increase in pulse of > 20 beats/minute
- If determined necessary by the study physician; e.g. diarrhea with nausea/vomiting and unable to drink enough to keep up with output, or other reason

11.5.2 Visit 9-10 (Day 5-6 post challenge)

(Unless discharged early from the inpatient unit)

- Changes regarding AEs and concomitant medication will be captured.
- If not started sooner, antibiotic therapy will be started on Visit 9 (Day 5 post challenge). Subjects will remain on the unit until discharge criteria are met.
- Vital Signs (VS) will be assessed at least thrice daily: in the morning, in the afternoon and in the evening.
- A clinician will conduct a daily medical interview and focused PE to assess health status, follow-up, monitor, and treat as indicated.

- All stools will be collected for weighing and grading. Once antibiotics are started, 3 stool samples will be collected daily for culture. If a subject is unable to provide a stool sample by 01:00 pm, he/she will be asked to obtain a rectal swab.

11.5.3 Visit 11 (Day 7 post challenge)

Visit 11 will be performed either as an inpatient visit (if subject has not yet been discharged) or as an outpatient visit (for subjects discharged earlier).

- Changes regarding AEs and concomitant medication will be captured.
- Subjects will have blood drawn for clinical chemistry and hematology (and immunological assays).
- Vital Signs (VS) will be assessed at least thrice daily: in the morning, in the afternoon and in the evening, if still inpatient, and once if already discharged.
- A clinician will conduct a daily medical interview and focused PE to assess health status, follow-up, monitor, and treat as indicated.
- All stools will be collected for weighing and grading until subject has had two negative stools for culture then no further stools for culture are required. If a subject is unable to provide a stool sample by about 01:00 pm he/she will be asked to provide a rectal swab (at least one up to 3 per day)
- Subjects discharged early will return for Visit 11 post-challenge and provide the requisite samples (stool, blood) as delineated in the Table of events (see 11.7).

11.5.4 Visit 12 (Discharge Visit)

- Routine discharge is scheduled for Day 8 post challenge. Two consecutive negative stool cultures for the challenge strain are required before discharge (can be collected on the same study day).
- Remaining doses of antibiotic will be given to the subject for self-administration.
- Changes regarding AEs and concomitant medication will be captured.
- VS at discharge will be recorded in the source documents.
- A clinician will conduct a focused PE to assess health status, follow-up, monitor, and treat as indicated.

Extension of the inpatient period might be required up to 10 days post challenge in case two consecutive negative stool cultures for the challenge strain are not available at Day 8 post challenge. Further inpatient visits will include assessments as described for Visit 9 above (see section 11.5.2).

11.5.5 Visit 13: Follow-up post challenge (Day 28 ± 2 post challenge)

Outpatient Follow-Up: challenge subjects will have outpatient follow up in the clinic. Procedures this day will include:

- Vital Signs (blood pressure, heart rate, and temperature)
- Review of con meds, AE's, complaints or procedures

- Optional Focused PE (based on symptoms)
- Stool collection for fecal IgA
- Blood for exploratory, immunology (IgG, IgA), and ALS
- Females will have urine pregnancy testing completed

11.5.6 Visit 14 (Day 180 +1/-14 days post vaccination): Follow up contact

Phone Call Follow Up: Subjects will be called to assess the occurrence of any serious emergent medical conditions, new onset of chronic illnesses and completion of the IBS survey.

11.5.7 Unscheduled Visit (s)

An unscheduled visit can be held at any time during the study if deemed necessary by the Investigator (e.g. follow-up on unexpected AEs or SAEs). Assessments performed at an unscheduled visit will be at the Investigator's discretion. Unscheduled visits and any procedures/assessments performed during such a visit (e.g. physical examination, laboratory test) should be documented in the source data.

11.5.8 Early Termination Visit

Subjects who terminate participation or who are withdrawn from the study prior to their Visit 13 will undergo the same investigations as for Visit 13 below during an Early Termination Visit, if possible. Every effort should be made to have discontinued subjects complete the study Early Termination (ET) Visit.

In case an ET Visit as described above is not possible or in case of early termination after Visit 13, a follow-up safety phone call should be made as soon as possible after termination to capture at least concomitant medications and AEs since the last study visit.

The reason for early termination should be clarified in as much detail as possible. If an AE was the reason for early study termination details on that specific AE(s) should be captured. The reason for discontinuation will be recorded on the eCRF, and data collected up to the time of discontinuation will be used in the analysis and included in the clinical study report.

In the event of subject discontinuation due to an AE, clinical and/or laboratory investigations that are beyond the scope of the required study observations/assessments may be performed as part of the evaluation of the event. These investigations will take place under the direction of the Investigator in consultation with the Sponsor, and the details of the outcome may be reported to the appropriate regulatory authorities by the Sponsor.

11.5.9 End of study

Once Visit 14 has been completed, subjects will be considered as completed the study. When all subjects have completed Visit 13 the study's active portion is considered completed.

11.6 Procedures for Monitoring Subject Compliance

All study procedures are to be performed under the direction of the Principal Investigator at the study site, and thus, no separate procedures will be used to monitor subject compliance.

11.7 Trial and Events Schedule

Visit	V0[a]	V1	V2	V3	V4	V5	V6	V7	V8	V9, 10 [a)]	V11	V12	V13	V14
Timing	Day -60 to -1	Day 1	Day 8	Day 15	V3 + 1	V4 + 1	V4 + 2	V4 + 3	V4 + 4	V4 + 5, +6	V4 + 7	V4 + 8	V4 + 28 days	Month 6
Time windows	-60 to -1	0	0	0 to +3	0 to +3	0	0	0	0	0	0	0	-2 to +2	-14 to -1
Visit type	Screening	Outpatient visit, First vaccination	Outpatient visit, Second vaccination	In-patient	In-patient	In-patient	In-patient	In-patient	In-patient	In-patient	In-patient/ Outpatient	Discharge	Out-patient visit	Safety Follow-up Phone call
Informed Consent	X													
Demography [b)]	X													
Eligibility criteria	X	X	X	X	X									
Pregnancy test	X (serum or urine)	X (urine)	X (urine)	X (serum, urine)									X (urine)	
Medical history	X	X												
Drug screen	X													
Hematology/ Serum Chemistry	X			X							X			
Admission to inpatient unit/discharge				X	X	X	X	X	X	X	X	X		
Physical exam	X			X										
Focused physical exam		X	X		X	X	X	X	X	X	X	X	(X)	
Vital signs	X	X	X	X	X	X	X	X	X	X	X	X	X	
ECG				X										
Symptoms/AEs			X	X	X	X	X	X	X	X	X	X	X	X

Visit	V0	V1	V2	V3	V4	V5	V6	V7	V8	V9, 10 [a]	V11	V12	V13	V14
Timing	Day -60 to -1	Day 1	Day 8	Day 15	V3 + 1	V4 + 1	V4 + 2	V4 + 3	V4 + 4	V4 + 5, +6	V4 + 7	V4 + 8	V4 + 28 days	Month 6
Time windows	-60 to -1	0	0	0 to +3	0 to +3	0	0	0	0	0	0	0	-2 to +2	-14 to -1
Memory Card		dispense	collect/ dispense	collect										
IBS Survey	X													X
Concomitant therapy		X	X	X	X	X	X	X	X	X	X	X	X	
Randomization		X												
Vaccination		X	X											
Bacterial inoculation					X									
Antibiotic administration						(X) [c]	(X) [c]	(X) [c]	(X) [c]	X	X			
Stool collection [e]	X	(X) [d]		X	X	X	X	X	X	X	X	X	X	
Grading and weighing					X	X	X	X	X	X	X	X		
Stool culture					X	X	X	X	X	X	X	X		
CFU quantification in stool							X		X					
Fecal IgA	X	X [d]		X							X		X	
Serum IgG		X		X							X		X	
Serum IgA		X		X							X		X	
ALS		X		X							X		X	

a) optional visits
b) include date of birth, height, weight, BMI, gender and race
c) antibiotic treatment will be started according to criteria described in Section 11.5.1
d) if not collected at Visit 0
e) Stool samples will be collected for assays as specified in the laboratory study of event schedule and as per written study specific procedures. A subset of these samples, during high shedding points, will be reserved for the later validation and development of bacteriological assays for shedding of ETEC and other organisms. Additionally, stool samples will be obtained to assess for exploratory endpoints to include microbiome, PCR, other immunology assays and transcriptomics.

12. ETEC DISEASE-SPECIFIC EXPECTED EVENTS

ETEC Disease-specific expected events will be recorded after challenge (Visit 4) up to Visit 12. For that specific time-window, these events will not be defined as adverse events (see also section 13.1.3). ETEC Disease specific events include the following symptoms:

1. Diarrhea
2. Fever
3. Nausea
4. Bloating
5. Vomiting
6. Generalized Myalgia
7. Arthralgia
8. Abdominal pain
9. Abdominal cramping
10. Malaise
11. Headache
12. Loss of appetite
13. Dizziness

Severity of ETEC disease-specific expected events will be graded in identical manner to adverse events (section 13.2.1.4)

13. ASSESSMENT OF SAFETY

13.1 Definitions

13.1.1 Adverse Events

An adverse event (AE) is defined as any untoward medical occurrence in a subject associated with the use of an investigational product administered in humans, whether or not considered product related. All new abnormalities or any exacerbation in intensity or frequency (worsening) of a pre-existing condition during or after the first vaccination will be documented as AEs. Pre-existing conditions will not be considered Adverse Events, see Section 13.3.1.

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

13.1.2 Serious Adverse Event

An adverse event is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Outcome is fatal/results in death;
- Is life-threatening – defined as an event in which the subject was, in the judgment of the Investigator, 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 results in prolongation of an existing hospitalization;
- Results in persistent or significant disability/incapacity (ie, a substantial disruption of a person's ability to conduct normal life functions);
- Results in congenital anomaly/birth defect;
- Is a medically important condition – a medical event that may not be immediately life-threatening or result in death or require hospitalization but may jeopardize the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the definitions above. This definition also applies to progression of disease leading to a serious outcome.

In case of hospitalization or prolonged hospitalization for diagnostic or elective medical procedures that were planned prior to first vaccination to treat a pre-existing condition which did not change in severity, neither the condition leading to the hospitalization or prolonged hospitalization, nor the medical procedure itself need to be reported as an SAE. In this case, the underlying diagnosis or condition should be reported in the medical history section of the eCRF and the corresponding medical procedure should be documented as a comment to the underlying diagnosis or condition in the eCRF's medical history section.

Unexpected adverse event: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed.

The Sponsor will classify the SAEs as either expected or unexpected:

- **Expected:** An AE that is listed in the current Investigator's Brochure (IB);
- **Unexpected:** An AE that is not listed in the current IB or it differs because of greater severity or greater specificity.

13.1.3 Untoward Medical Occurrences Not Considered Adverse Events

Each untoward medical occurrence experienced before the first vaccine exposure (for example, from the time of signed informed consent up to but not including the first vaccine exposure) will be described in the medical history as a pre-existing condition.

For the study period from challenge until discharge from inpatient unit (Visit 4 to Visit 12), pre-defined ETEC disease-specific expected events (see section 12) will be reported

separately (e.g. for the primary and secondary endpoints of the study). Consequently, ETEC disease-specific expected events with start date within this particular study period will not be defined as adverse events for the safety analysis.

13.1.4 Independent Research Monitor

The research monitor, in accordance with JHBSPH guidelines, will have the following responsibilities:

- Evaluate ongoing safety data and make recommendations in order to ensure subjects safety as required
- Be available for consultation by the clinical investigative team through the period of the clinical study in which there is an interaction with human subjects
- Be available to review all SAEs and other unanticipated problems involving risk to subjects
- Be available to discuss SAEs and significant safety issues
- Provide clinical advice, in accordance with the study protocol, on the clinical management of subjects. This advice may include, but is not limited to
- Decisions on "borderline" laboratory values and eligibility for enrollment
- Confirmation and discussion of treatment decisions for difficult clinical situations
- Must document all clinical decisions including date, time and signature
- Must communicate all decisions to the study Principal Investigator and other study investigators, which must be stored with subject source documents

13.2 Collection, Documentation and Assessment of Adverse Events

13.2.1.1 Solicited adverse events after vaccination

Subjects will be provided with a Memory Card to collect predefined events compiled from the safety profile of Dukoral as well as ETEC disease specific expected events:

1. Diarrhea
2. Fever
3. Nausea
4. Bloating
5. Vomiting
6. Generalized Myalgia
7. Arthralgia
8. Abdominal pain
9. Abdominal cramping

10. Malaise
11. Headache
12. Loss of appetite
13. Dizziness
14. Fatique
15. Urticaria
16. Rash
17. Chills

Solicited AEs are per definition regarded as related to the IMP. Only in case solicited AEs are serious the investigator will perform more detailed assessments and will document them in the eCRF AE log.

Severity of solicited AEs will be assessed as described in Section 13.2.1.4.

Assessments by the subject should occur about the same time each day, in the evening. The subject will be properly instructed on the reporting requirements and how to complete and use the subject memory cards and thermometer.

The memory card is to be collected by study staff at indicated visits. The Investigator or designee will review and discuss the memory card with the subject, ask about AEs occurring since the last visit and both the subject and Investigator have to sign the memory card to ensure completeness and reliability of self-reporting. Entries in the memory card shall be evaluated and graded for severity.

The memory card will serve as source documentation. Entries in the memory card will be transcribed to the eCRF. Non-completion of the memory card will be considered a protocol deviation, however, errors in completion will be reviewed with the subject and updated as needed and not considered protocol deviations.

In addition to solicited adverse events, unsolicited AEs (section 13.2.1.3) as well as any new concomitant medication or changes in medication taken after vaccination will be captured.

13.2.1.2 Solicited events after challenge

ETEC Disease-specific expected events will be recorded after challenge (Visit 4) up to Visit 12. However, for that specific time-window, these solicited events will not be defined as adverse events (see also section 12 and section 13.1.3) unless causal relationship with the challenge strain may be excluded.

13.2.1.3 Unsolicited adverse events

The Investigator should enquire about AEs during study visits. Clinically relevant laboratory parameter changes constitute unsolicited AEs, too, unless they are considered a symptom of an underlying AE or part of a syndrome that is reported as AE (e.g. presence of blood cells in urine in a person diagnosed with urinary tract infection). In addition, symptoms noted

during the symptom-driven physical exams (unless already covered by an AE) constitute AEs.

All AEs need to be documented in the respective AE section of the eCRF during every study visit (Visits 1 to 14 or unscheduled visit(s), if applicable), regardless of their source, open question to subject, laboratory parameters, focused physical examination).

Any symptom is regarded as a separate AE. However, if the Investigator considers several symptoms to be in the context of one underlying diagnosis, the Investigator may merge these symptoms into one single appropriate AE. The AE term entered into the eCRF should contain all symptoms summarized to one unifying diagnosis (e.g. "Influenza", as opposed to "fever and headache").

The Investigator will follow-up on each AE until it is resolved or until the medical condition of the subject is stable. All relevant follow-up information will be reported to the Sponsor until the end of the study for each subject. SAEs ongoing at the time of Visit 13 will be followed until resolution or achievement of stable clinical conditions, latest until completion of Day 180 follow-up call.

Any safety event (Serious emergent medical conditions, new onset of chronic illnesses) that is identified at the last assessment (Visit 14) must be recorded on the AE eCRF with the status of the safety event noted. All serious suspected adverse reactions and serious adverse reactions will be followed until resolution or until the patient is medically stable.

The following information will be documented for each AE: severity, causality (relationship), outcome, seriousness, action taken to treat AE, action taken on IMP and start and stop dates.

Each AE from vaccination until study completion/termination will be described on the AE eCRF using the medical diagnosis (preferred), symptom, or sign, in standard medical terminology in order to avoid the use of vague, ambiguous, or colloquial expressions (see definition in Section 13.1.1). AEs will be evaluated by the Investigator for:

1. Seriousness as defined in Section 13.1.2
2. Severity as defined in Section 13.2.1.4
3. Causal relationship to vaccine, challenge or antibiotic treatment as defined in Section 13.2.1.5

13.2.1.4 Severity

The following symptoms will be rated according to the following adjusted FDA Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials (September 2007) as described in Table 3. Any grade 4 reaction should be reported as an SAE (see Section 13.1.2).

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Diarrhea*	1-3 loose stool or ≤ 400g in any 24-hour period.	4-5 loose stools or 401 to 800 g in 24 h;	at least 6 loose stools or >800 g in 24 h;	ER visit or hospitalization
Fever**	38.0 – 38.4 °C 100.4–101.1 °F	38.5 – 38.9 °C 101.2–102.0 °F	39.0 – 40.0 °C 102.1–104 °F	> 40.0 °C >104 °F
Nausea	No interference with activity	Some interference with activity	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Vomiting	No interference with activity or 1-2 episode(s) within a 24-hour period	Some interference with activity or > 2 episodes within a 24-hour period	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization

* During the inpatient period, each stool passed is collected, weighed, and graded as follows: grade 1, firm formed; grade 2, soft formed; grade 3, viscous opaque liquid or semiliquid which assumed the shape of the container; grade 4, watery opaque liquid; and grade 5, clear watery or mucoid liquid. Stools defined as grade 3, 4, or 5 are considered to be loose and to potentially contribute to an episode of diarrhea. Episodes of grade 3 to 5 stools that do not meet any of the above definitions are classified as loose stools and are not captured as adverse events. In calculating the total number and weight of diarrheal stools following challenge only stools which contributed to an episode of diarrhea according to these definitions will be included. An episode will be considered complete after 24 without a loose stool. During the outpatient period, loose stools will only be graded and assessed by the subject. No stools will be weighed during the outpatient period.

**Oral temperature; no recent hot or cold beverages or smoking. ****Grading based on Common Terminology Criteria for Adverse Events (CTCAE), NIH, v4.03, 2010.

Table 3 Severity Grading of Systemic Reactions – Vaccine Specific Criteria

For other events (including other solicited events such as abdominal pain/cramps, bloating, arthralgia, malaise, loss of appetite) the investigator will assess the severity of AEs using his/her clinical expertise and judgment based on the most appropriate description below:

Mild (Grade 1)	A type of adverse event that is usually transient and may require only minimal treatment or therapeutic intervention. The event does no generally interfere with usual activities of daily living.
Moderate (Grade 2)	A type of adverse event that is usually alleviated with additional specific therapeutic intervention. The event interferes with usual activities of daily living, causing discomfort but poses no significant or permanent risk of harm to the subject.
Severe (Grade 3)	A type of adverse event that interrupts usual activities of daily living, or significantly affects clinical status, or may require intensive therapeutic intervention

Table 4: Severity Grading of other events

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

13.2.1.5 Causality

Causality is a determination of whether there is a reasonable possibility that the IMP administration is etiologically related to/associated with the AE.

For AEs, the Investigator or designee will assess the causal relationship between the IMP and the AE using his/her clinical expertise and judgement.

The site PI should consider the following question when assessing causality of an AE to study product:

Is there a reasonable possibility that the product caused the event?

Reasonable possibility implies there is evidence that the study product caused the reported event. An affirmative answer designates the event as a suspected adverse reaction, and the AE is therefore considered "related." If the answer is no, then the AE is considered "unrelated."

13.2.1.6 Follow up and Outcome of Adverse Events

Investigators are required to follow AEs and SAEs to resolution, even if this extends beyond the prescribed reporting period. Resolution is the return to baseline status or stabilization of the condition with the probability that it will become chronic or will resolve. The AEs and SAE outcomes will be reported to the sponsor.

Investigators are not obligated to actively seek AEs and SAEs in former subjects; however, if an AE or SAE, considered to be related to the investigational product is brought to the attention of the investigator *at any time* until closure of the study, the event will be reported.

The investigator will assess the outcome of all unsolicited AEs (including SAEs) recorded during the study as:

- Recovered/resolved without sequelae.
- Recovered/resolved with sequelae.
- Recovering/resolving.
- Not recovered/Not resolved.
- Fatal (SAEs only).

13.2.1.6.1 Body Temperature Measurement

From first vaccination (Visit 1) until Visit 3, 7 days post 2nd vaccination, the subject should measure his/her body temperature orally once every evening. To optimize the comparability of the documented body temperatures, all subjects will be provided with a digital oral thermometer and be instructed in its use. The subjects may keep the thermometer after study termination.

If fever (oral body temperature ≥ 38.0 °C/100.4 °F) occurs, this should be confirmed with another check within the hour prior to use of antipyretics and avoiding eating, chewing gum, strenuous activity, and smoking. If not confirmed, it will not be considered an AE. If confirmed, subject should call the study staff and take temperature every 4 to 8 hours until it returns to normal (< 38.0 °C/100.4 °F). All abnormal (out of range 35.0°C to 37.9°C) body temperature measurements including the date and time should be recorded. In case of fever, the subject should record all fever measurements including the first value that shows a return to normal body temperature.

Abnormal body temperature measurements will be recorded by the Investigator in the eCRF. If more than one body temperature value is recorded in the memory card for a given day, the highest daily temperature reading will be recorded in the eCRF.

Body temperature measurements will be analyzed according to the FDA Guidance on Toxicity Grading Scales for Healthy Adult and Adolescent Subjects Enrolled in Preventive Vaccine Clinical Trials (Food and Drug Administration) for data analysis as described in Table 3 above.

13.3 Medical, Medication, and Non-Drug Therapy History

13.3.1 Medical history

At screening, the subject's medical history will be described for the following body systems or surgery and start and end dates, if known: head, eyes, ears, nose, and throat; respiratory; cardiovascular; gastrointestinal; musculoskeletal; neurological; endocrine; metabolic; hematopoietic/ lymphatic; dermatological; and genitourinary.

Preexisting conditions will be regarded as medical history if present before entry in to the study, and manifest with the same severity, frequency, or duration after vaccine exposure, those will not be recorded as AEs. Furthermore, routine health checks required due to pre-

existing diseases will not be recorded. However, when there is an increase in the severity or duration of a preexisting disease, the event must be described on the AE CRF page.

13.3.2 Concomitant Medications and Non-Drug Therapies

All medications taken or change in medications taken during the active portion of the study should be collected from all subjects, and recorded on the appropriate eCRF, including IVF's. In addition to product (generic) name, the dose, indication, route of administration and frequency as well as the start and end date of treatment will be documented.

In addition, medications to treat SAEs will be reported to the Sponsor on SAE Report Forms as described in Section 2. In context of this study, information on non-drug therapies will only be collected in relation to SAEs.

The following medications are **not permitted** if administered within the specified study periods:

- Any blood products or immunoglobulins during the active course of the study (through Day 44);
- Immunosuppressive therapies (e.g. systemic or high dose inhaled [>800 µg/day of beclomethasone dipropionate or equivalent] corticosteroids, radiation treatment or other immunosuppressive or cytotoxic drugs) during the course of the study (unless such treatment has to be administered in an emergency situation);
- Prophylactic administration of antipyretics within 4 hours prior to and during the first 24 hours after each vaccination;
- Other vaccinations (except in medical emergencies such as tetanus or rabies exposure) within 8 weeks (for live vaccines) and 4 weeks (for inactivated vaccines), respectively prior to vaccination in this study;
- Subjects are requested to refrain from donation of blood, blood fractions and plasma within 30 days prior first vaccination and for the entire active study duration

For documentary purposes, any of the treatments listed above (including emergency treatment) given within these time periods requires special documentation and is to be documented as a protocol deviation.

Additionally, medications that are not permitted prior to study enrollment, resulting in exclusion from the study, are reflected in the exclusion criteria in Section 9.3.

13.4 Vital Signs

Vital signs (VS) will include body temperature (°C / °F) measured orally, heart rate (beats/min), and systolic and diastolic blood pressure (mmHg) while at rest position will be documented.

VS will be measured at screening (Visit 0) and at each vaccination visit (Visit 1 and 2) and are to be recorded before the vaccination is given and after an observation period of at least 30 minutes following each vaccination.

Starting at Visit 4, VS will be assessed thrice daily until the subject's discharge.

For each abnormal vital sign value, verified upon repeat within 20 minutes, the Investigator will determine whether the value is considered an AE (see definition in Section 13.1.1). If assessed as an AE, the medical diagnosis (preferably), symptom, or sign, will be recorded on the AE CRF (only the confirmatory vital sign will be recorded). Additional tests and other evaluations required to establish the significance or etiology of an abnormal result or to monitor the course of an AE should be obtained when clinically indicated. Any abnormal value that persists should be followed at the discretion of the Investigator.

VS measured prior and post vaccination as well as prior and post challenge will be captured in the eCRF. VS measured at other time points will only be transferred to the eCRF if abnormal.

13.5 Physical Examinations

At screening (Visit 0) and at Visit 3, a physical examination will be performed on the following body systems: general appearance, head and neck, eyes and ears, nose and throat, chest, lungs, heart, abdomen, extremities and joints, lymph nodes, skin, and neurological. Findings on exam will be described as normal, abnormal in the opinion of the Investigator or not clinically significant (NCS).

A focused physical exam will be performed at all visits except Visit 0 and Visit 3 (as described in Trial and Events Schedule, 0). This will include: general appearance, head and neck, throat, chest, lungs, heart, abdomen and gross neurological exam.

Abnormal conditions detected at screening or prior to vaccination at Visit 1 will be recorded as medical history. At all other study visits, if a new abnormal or worsened abnormal pre-existing condition is detected, the condition will be recorded as an AE.

13.6 Clinical Laboratory Parameters

Blood will be obtained for assessment of clinical laboratory parameters as outlined in the Trial and Events Schedule (see Section 11.7). Parameters will be analyzed by local laboratories according to the applicable laboratory SOP:

Clinical chemistry (BMP) **(5 mL)**	sodium, chloride, bicarbonate, potassium, creatinine, blood urea nitrogen, glucose and alanine aminotransferase (ALT)
Hematology panel **(3 mL)**	Hemoglobin, hematocrit, CBC count, differential WBC count (basophils, eosinophils, lymphocytes, monocytes, neutrophils), platelets
HBsAg/ HCV / HIV test **(15 mL)**	A positive HIV test obtained by ELISA will have to be confirmed by a second method [e.g. Western blotting or PCR] HIV/HBsAg/HCV test need to be performed within 30 days prior to Visit 0, to establish negative status eligibility for the study

Urine Toxicology	Urine toxicology for drugs of abuse (opioids, benzodiazepines). Marijuana is not an exclusionary criterion.
Pregnancy test (Serum 5 mL)	A β-hCG urine pregnancy test will be performed on all female subjects of childbearing potential prior to each vaccination, on the day of admission and 28 days post challenge (Visits 1, 2, 3 and 13). A serum pregnancy test (3 mL) will be performed at the Visit 0 and 3.

13.6.1 Assessment of Laboratory Values

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. For the individual toxicity criteria refer to Section 13.6.2.

Laboratory assessments for which no severity grading is described in Section 13.6.2 are graded as described in Section 13.2.1.4 upon investigator's judgment.

The Investigator's assessment of each abnormal laboratory value, based on the toxicology tables, including its clinical significance, is to be recorded in the source and, if considered clinically significant, in the eCRF:

- Abnormal laboratory assessments that are considered clinically relevant, in the opinion of the Investigator, need to be documented and assessed further for severity according to the toxicity grading scale provided in Table 5 and Table 6, causality.
- Abnormal laboratory assessments that are considered a symptom of an underlying AE or part of a syndrome that is reported as AE (e.g. presence of blood cells in urine in a person diagnosed with urinary tract infection) do NOT additionally need to be documented as AE, but a respective comment should be added to the underlying AE.

Additional tests and other evaluations required to establish the significance or etiology of an abnormal laboratory result or to monitor the course of an AE should be obtained when clinically indicated. Any abnormal value that persists should be followed at the discretion of the Investigator.

13.6.2 Reference Ranges and Adverse Event Coding for Clinical Laboratory Parameters

Test	Quest Normal	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening
Hemoglobin (g/dL)	M: LLN = 13.2 F: LLN = 11.7	M: 11.0-12.5 F: 9.5-10.7	M: 9.0-10.9 F: 8.0-9.4	M: <8.9 F: <7.9	Life Threatening
Neutrophils (cells/mm3)	1,500-7,800	750-999	500-749	<500	Life threatening

Leukocytes (white blood cells)	3,800-10,800				
Leukopenia		2,500-3,300	1,500-2,499	1,000-1,499	< 1,000
Leukocytosis		11,500-13,000	13,001-15,000	>15,000	Life threatening
Lymphocytes (cells/mm3)	850-3,900	750-849	500-749	250-499	< 250
Eosinophils (cells/mm3)	15-500	551-1,500	1,501-5,000	> 5,000	Hypereosinophilic
Platelets decreased – 103/mm3	140,000-400,000	100,000-125,000	75,000-99,000	25,000-74,999	< 25,000

Table 5: Reference Ranges and Adverse Event Coding for Hematology

Test	Normal	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Sodium	135-146 (mmol/L)				
Hyponatremia		132-134	130-131	125-129	< 125
Hypernatremia		147-148	149-150	151-152	> 152
Potassium	3.5-5.5 (mmol/L)				
Hypokalemia		3.3-3.4	3.1-3.2	2.9-3.0	< 2.9
Hyperkalemia		5.6-5.7	5.8-5.9	6.0-6.1	> 6.2
Chloride	98-110 mmol/L	90-97	80-89	70-79	<70
Bicarbonate	22-29 mmol/L	19-22	16-18	12-15	<12
Glucose, Random	65-139 (mg/dL)				
Hyperglycemia		140-155	156-200	> 200	Insulin requirements
Hypoglycemia		60-64	55-59	45-54	< 45
SGPT/ALT (elevation)	M: 9-60 U/L F: 6-40 U/L	M: 61-150 F: 41-100	M: 151-300 F: 101-200	M: 301-600 F: 201-400	M: > 600 F: > 400
BUN (elevation)	7-25	26-28	29-31	> 31	Requires dialysis

Creatinine (elevation)	M: 0.7-1.4 F: 0.5-1.1	1.1-1.3 x ULN	1.4-1.8 x ULN	1.9-3.4 x ULN	≥3.5 x ULN

Table 6: Reference Ranges and Adverse Event Coding for Clinical Chemistry Parameters

13.7 SAE Reporting

13.7.1 Site Investigator:

All SAEs must be reported promptly (within 24 hours) to the sponsor as per 21 CFR 312.64. Further, the investigator should comply with relevant study site SOPs on reporting SAEs.

All serious events should be entered into the data system within 24 hours of identification. If there are technical difficulties when entering the event into the EDC system, the SAE will be reported to EMMES, and these numbers will be provided in the Manual of Operations. All information reported by fax will need to be entered in the data system when it is available.

All SAEs must be reported immediately by the investigator without filtration, whether or not regarded as possibly attributable to the test articles, placebo, or antibiotic. This initial notification will include minimal, but sufficient information including the test articles, and date of onset, severity and causality. The investigator will not wait for additional information to fully document the event before notifying. The initial report is then to be followed by submission of a follow up report as soon as possible but not more than 3 calendar days past the initial report, detailing relevant aspects of the AE in question. All investigator actions and event outcomes must also be reported immediately. Hospital records and autopsy reports will be obtained if applicable.

Investigators must follow all relevant regulatory requirements as well as specific policy at each institution regarding the timely reporting of SAEs to the local IRB and Research Monitor.

Reporting to the sponsor does not fulfill the investigator's duty to report all unanticipated problems involving risk to human subjects or others to the IRB. The PI will notify the local IRB, Emmes, and the Research Monitor.

13.7.2 Emmes Medical Monitor:

The Emmes Medical Monitor is notified immediately via email of any SAE when it is reported in data system. This notification will mark as Day 0 for safety reporting timelines. The MM reviews the SAE in one business day of receiving SAE notification.

The Emmes Medical Monitor is responsible for notifying the Sponsor and will do so simultaneously with the reporting to the clinical database. The Emmes Medical Monitor will review each SAE report and will determine whether the SAE must be reported to FDA/regulatory authorities on an expedited basis. The final decision for disposition regarding reporting to the FDA/regulatory authorities' rests with the Sponsor. The IND Sponsor or their designee is responsible for submitting the SAE reports to FDA/regulatory authorities. The Coordinating Center will maintain copies of any SAE reports submitted to FDA/regulatory authorities by the Sponsor.

13.7.3 Independent Research Monitor:

Please refer to section 13.1.4.

13.7.4 Sponsor:

In order to comply with regulations mandating sponsor notification of serious unexpected suspected adverse reactions are to be reported within 15 days; 7 days are applicable for unexpected fatal or life-threatening suspected adverse reactions to the FDA. The sponsor will report unexpected SAEs associated with the use of the challenge strain to the FDA as specified at 21 CFR 312.32 (c).

Sponsor	Valneva Austria GmbH Email: [redacted]
Emmes Medical Monitor	[redacted]
Institutional Review Board	JHSPH IRB Office [redacted]
Independent Research Monitor	[redacted]

Table 7: Study Contacts for Reporting Serious Adverse Events

13.8 Pregnancy Reporting

Women must not become pregnant through Day 44 of the study. If a subject becomes pregnant up to Day 44, she must immediately inform the Investigator.

Reporting requirements start with the first vaccination. All pregnancies will be followed up for three months after delivery or termination of the pregnancy. Any effect on either mother or fetus should be determined. A pregnancy which led to a congenital anomaly/birth defect must be followed-up by the Investigator longer or until resolution which will be decided on individual basis and in accordance with the Sponsor.

The Investigator should report pregnancies to Emmes and the Sponsor within 24 hours of being notified using the Pregnancy Report Form. Reporting procedures are similar to SAE reporting procedures (contacts and processing). Pregnancy is not considered an SAE. Routine hospitalization for labor/delivery is not considered a SAE. However, if a seriousness criterion applies in addition to the pregnancy (e.g. hospitalization due to a pregnancy complication, congenital anomaly/birth defect) the pregnancy qualifies as an SAE. In such case a Pregnancy Report Form and an SAE Report Form have to be filled out.

14. ASSESSMENT OF IMMUNOGENICITY

Immunological assessments will be conducted by:

[redacted]
CIR Enteric Research Laboratory (JHSPH)
[redacted]

15. STATISTICS

15.1 Sample Size and Power Calculations

Up to 34 subjects will be randomized 1:1 to receive either VLA1701 or placebo, 30 subjects will be challenged.

There was no formal sample size calculation for this pilot study rather study size was planned considering site capabilities and reference of studies previously described for challenge studies. The primary objective is to gain information on disease induced by the challenge strain in the placebo group in order to confirm published characteristics of the challenge strain and to appropriately design future studies based on the findings of this pilot study (e.g. If 7 or more Subjects in Placebo group develop diarrhea, a diarrhea rate induced by the challenge strain of 70% or above cannot be excluded with one-sided 95% CI).

15.2 Datasets and Analysis Cohorts

The safety analysis dataset contains all subjects who entered into the study and received at least one vaccination. Subjects will be analyzed as treated.

The primary analysis will be done in the challenge population which will be defined as subjects who received two doses of VLA1701 and the challenge dose.

Subjects will be analyzed according to the treatment group they had been allocated to, rather than by the actual treatment they received. Since this is a pilot study and the main objective is to gain information on disease induced by the challenge strain in placebo subjects, if any subject receives the wrong product then a sensitivity analysis will be performed where subjects will be analyzed as treated.

15.3 Handling of Missing, Unused, and Spurious Data

Only subjects for whom data are available will be included in the statistical analysis. Missing values will neither be replaced nor estimated.

15.4 Methods of Analysis

The primary analysis will be evaluated in the challenge population (i.e. all subjects who received two doses of the vaccine and the challenge dose). All endpoints on diarrhea after challenge (incl. the primary endpoint) will be calculated based on the protocol defined criteria (section 13.2.1.4). In addition, sub-analysis by grading diarrhea severity after challenge solely on stool volume alone (i.e. not taking into account the number of stools) will be done for all diarrhea endpoints. The definition of diarrhea in the protocol for the primary endpoint is based on multiple recent trials with numerous ETEC strains including the strain of this study. In particular, studies with H10407 (a CFA/I, LT+, ST+ strain), B7A (a CS6, LT+, ST+ strain), LSN03-016011/A (a CS17, LT+ strain) have all utilized a primary endpoint of diarrhea both the frequency and volume of loose stool to assess the efficacy of prophylactic products against ETEC challenge (NCT03040687, NCT02773446, NCT01922856, NCT01060748, NCT00435526). Additionally, because this study is designed to provide a proof of concept for VLA1701 in preventing diarrhea in travelers, an endpoint that would be of utmost

importance for a travel population and consistent with the endpoint utilized in studies of travelers' diarrhea is used (Alajbegovic et al (2012). Syst Rev 1(1): 39.; Porter et al (2017) Mil Med 182(S2): 4-10; 3. Shah et al (2009). Am J Trop Med Hyg 80(4): 609-614; Sack et al (2007) Vaccine 25(22): 4392-4400 Steffen et al (2013). J Travel Med 20(6): 374-379. Behrens et al (2014). Lancet Infect Dis 14(3): 197-204). Diarrhea cases will be reviewed by a blinded, independent Clinical Endpoint Committee (see 17.8) who will assess each subject's contribution to the primary endpoint. ETEC disease-specific events (including severe to moderate diarrhea after challenge for the primary endpoint) will be reported as "ETEC Disease-specific Expected Events" (as defined in section 12), after challenge (Visit 4) up to discharge from inpatient period (Visit 12). These events will not be defined as adverse events but will be reported separately as number and percentages for the inpatient period Visit 4 to Visit 12 (i.e. up to 7 days after challenge) and will be compared between the study arms using Fisher's exact test.

Disease severity score will be calculated as described in Porter, et al; An Evidenced-Based Scale of Disease Severity following Human Challenge with Enteroxigenic Escherichia coli; Plos one, 2016. Differences in the disease scores between groups will be compared using a Student's t-test with a 2-sided alpha = 0.05.

All subjects entered into the study, who receive at least one dose of VLA1701, will be included in the safety analysis. The number and percentage of subjects with solicited adverse reactions up to 7 days after each vaccination and unsolicited AEs during the entire study period, including the inpatient period, visit 13 (1 month after challenge) and visit 14 for SAEs (6 months after first vaccination), will be presented for each study arm overall and by body system / preferred term and will be compared using Fisher's exact test.

Changes in laboratory values and the frequency of clinically relevant abnormal values will be analyzed descriptively.

The exploratory immunogenicity analysis will be done on collected Serum, PBMCs and stool samples during the study.

Data Analysis

All tests and CIs will be two-sided unless stated otherwise. Evidence of statistically significant differences of endpoints will require a p-Value of 0.05 or less in a two-sided comparison.

An interim analysis including the primary endpoint analysis will be performed after all subjects have gone through challenge and have been released from the inpatient facility (Visit 12). A final analysis will be performed once the last subject has completed the study, i.e. Month 6 (Visit 15) and a final clinical study report will be compiled

16. ETHICS AND REGULATORY ASPECTS

16.1 Compliance Statement

This study will be conducted in accordance with this protocol, current ICH/GCP guidelines, and with the applicable national and local regulatory requirements.

16.2 Institutional Review Board (IRB) and Regulatory Authorities

Before enrollment of healthy subjects into this study, the protocol, informed consent form, any promotional material/advertisements, and any other requested information will be reviewed and approved by the IRB and applicable regulatory authorities in accordance with local requirements. The study will commence only upon the Sponsor's receipt of approval from the IRB.

If the protocol and/or any other information given to the subject is/are amended, the revised document(s) will be reviewed and approved by the IRB and applicable regulatory authorities in accordance with local requirements, where applicable. The protocol amendment will only be implemented upon the Sponsor's receipt of approval. Amendments that are intended to eliminate an apparent immediate hazard to subjects may be implemented prior to receiving IRB and authority approval. However, in this case, approval must be obtained as soon as possible after implementation.

16.3 Subject Information and Informed Consent

It is the Investigator or designees' responsibility to obtain freely given written informed consent from the subject before the subject is exposed to any study-related procedures, including screening tests for eligibility.

The informed consent form will include a comprehensive explanation of the proposed treatment without any exculpatory statements, in accordance with the elements required by ICH GCP and applicable regulatory requirements. Healthy subjects will be allowed sufficient time to consider participation in the study after having the nature and risks of the study explained to them. By signing the informed consent form, healthy subjects agree that all evaluations required by the study will be completed, unless they withdraw voluntarily or are terminated from the study for any reason.

The Investigator/designee will explain that the subjects are completely free to refuse to enter the study or to withdraw from it at any time, without any prejudice and need for justification. The subjects will be informed that representatives of the Sponsor and health authority inspector may review their source records, and that these persons are bound by confidentiality obligations.

The subject will be given a copy or a second original of the ICF. An original of the signed and dated ICF must be retained in the site's records, and is subject to inspection by representatives of the Sponsor or representatives from regulatory agencies.

The Sponsor will provide to the Investigator in written form any new information that significantly bears on the subjects' risks associated with study vaccine exposure. The informed consent form will be updated, if necessary. This new information and/or revised

informed consent form, that has been approved by the applicable IRB and regulatory authorities, where applicable, will be provided by the Investigator to the subjects who consented to participate in the study.

16.4 Subject compensation

Volunteers will be compensated for their time and effort in this study. Compensation will be provided only for completed study procedures designated for compensatory payment. Volunteers will not be paid for missed outpatient visits, and may forfeit some or all of the bonus as a result of missed visits or non-compliance.

17. QUALITY CONTROL AND QUALITY ASSURANCE

17.1 Source Data and Records

Source data are defined as all information related to clinical findings, observations or other activities in the study, captured in original records or certified copies of original records. The Investigator will permit study-related monitoring, audits, IRB review and regulatory inspections, by providing direct access to source data/records. Source records should be preserved for the maximum period of time required by local regulations.

Source data entries must be made in accordance with local requirements. Signed and dated copies of the laboratory result reports have to be kept within the subject's source data file.

eCRFs will not be used as source data for any other variable.

17.2 Investigator's Responsibility

The Investigator will comply with the protocol (which has been approved by the IRB), ICH GCP, and applicable regulatory requirements. The Investigator is ultimately responsible for the conduct of all aspects of the study at the study site and verifies by signature the integrity of all data transmitted to the Sponsor. The term "Investigator" as used in this protocol, and in study documents refers to the Investigator or authorized study personnel whom the Investigator has designated to perform certain duties. Sub-investigators or other authorized study personnel are eligible to sign for the Investigator, except where the Investigator's signature is specifically required.

17.3 Training

The assigned CRA will ensure that the Investigator and study site personnel understand all requirements of the protocol, the investigational status of the vaccine, and his/her regulatory responsibilities as an Investigator. Training may be provided at an Investigator's meeting, at the study site, and/or by instruction manuals. In addition, the CRA will be available for consultation with the Investigator and will serve as the liaison between the study site and the Sponsor.

17.4 Monitoring

A designated monitor will check electronic system data and source data at regular intervals throughout the study to verify completeness, accuracy and consistency of the data, protocol adherence, and adherence to GCP guidelines. The monitor will work according to the Monitoring Plan. The Investigator will cooperate with the monitor to ensure that any discrepancies identified are resolved.

17.5 Audit and Inspection

Upon request, the Investigator will make all study-related source data and records available to a qualified quality assurance auditor mandated by the Sponsor or to regulatory inspectors.

The main purposes of an audit or inspection are to confirm that the rights and welfare of the subjects have been adequately protected, and that all data relevant for the assessment of safety and efficiency of the investigational product have appropriately been reported to the Sponsor.

17.6 Non-compliance with the Protocol / Protocol Deviations from the Protocol

Any deviations from the protocol will be tracked, actions defined, as feasible, and reviewed for the study interims analysis and the final analysis for assessment of their influence on the quality of the study analysis.

17.7 Confidentiality of Subject's Data

The Investigator will exercise all reasonable precautions within the constraints of the applicable regulatory requirements to maintain the confidentiality of subjects' identities. On exported electronic source data or any other documents submitted to the Sponsor, subjects will only be identified by subject number. Documents not for submission to the Sponsor, e.g. subject identification log and original ICF, will be maintained by the Investigator in strict confidence.

17.8 Independent Outcome Adjudication Committee

In an effort to obtain an unbiased determination of the efficacy outcomes, an independent outcome adjudication committee, the members of which will be blinded as to the treatment regimens of the subjects, will evaluate challenge outcome data after completion of the inpatient phase of the study.

The committee will be comprised of at least 3 individuals, independent of the study sponsor and investigative team, who are experts on diarrheal illness case identification and pathogen diagnosis. The committee will also include statistician data analyst who will lead and coordinate the committee but will have a non-voting role in deliberations. Details on composition of the CEC and data to be reviewed will be specified in the CEC Charter.

The committee voting members will review all potential efficacy-related cases and endpoint data. Among the committee's responsibilities, they will (1) review and confirm all primary endpoint cases; (2) review all protocol-specified entry criteria, adherence, and compliance issues to ascertain classification in the per-protocol and other study populations; and (3) provide guidance regarding secondary and other endpoint classifications to include agreement on objective criteria for classification of endpoints. Specific duties and responsibilities will be outlined by charter prior to the start of the study.

18. DATA HANDLING AND RECORD KEEPING

18.1 Information of Investigators

An IB containing all important data relating to the safe use of the IMP will be supplied to the Investigator prior to study start.

The Investigator will be kept informed on new relevant safety data as the study proceeds.

18.2 Electronic Case Report Forms (eCRFs)

18.2.1 Data Recorded Directly on Case Report Forms

Electronic Case Report Forms (eCRFs) will be used for this study. Data will be recorded directly onto source documents before documentation in the eCRF.

18.2.2 eCRF entries

eCRF entries and corrections will only be performed by study site staff authorized by the Investigator. Each user who will be entering data for this study will be trained by Emmes prior to being granted login credential for access to the data system for this study. On successful completion of data system training per Emmes' requirements, each user will receive their access credentials along with URL information linking to the Advantage eClinical® login interface. Each user's personal password must be kept confidential and must only be used by the person to whom it was assigned. For additional authorized users at the site, a new user account has to be requested to ensure that each entry/change can be attributed to the person who performed the entry/change.

All visit data need to be recorded in the Advantage eClinical as soon as possible. Outpatient visits 1, 2 and 13 will be entered within 3 days of each respective visit date. During the inpatient challenge period, data will be entered only after discharge unless there is an SAE. Stool data from the challenge period will be prioritized and entered within 4 weeks of Visit 12; the remaining data from the challenge period will be entered no later than 6 weeks after Visit 12.

18.2.3 Changes to eCRF data

Corrections may be requested as follows:

- Investigators' responses are checked as they are entered and are rejected if they do not fulfill quality criteria. A message will specify the type of error or syntax error and assist in its correction.
- If required, the CRA can ask for information to be corrected during monitoring.
- Computerized data-check programs and manual checks will identify clinical data discrepancies for resolution. Corresponding queries will be communicated to clinical site personnel.

All discrepancies will be resolved by the Investigator or by authorized staff, either through correction within the data system or, in some cases, by confirmation/validation of queried data point(s) against source documentation.

Corrections of eCRF data may be performed by authorized staff only. The person performing the changes in the eCRF is required to electronically confirm the changes made.

18.2.4 eCRF Entry Validation

The Investigator or the authorized delegate will thoroughly review the data on the eCRF, and will finally certify the contents of the eCRF by signature after completion of each subject.

18.2.5 Data collection

All visits and selected assessments as per protocol are entered into an interactive form. eCRFs will be source document verified following guidelines established before study onset and detailed in the Monitoring Plan. Maintenance of the study data system will be performed as needed throughout the study. Details pertaining to eCRF handling are provided study materials that will include study specific eCRF completion instructions.

18.3 Coding of Adverse Events, Drugs and Diseases

After data entry, AEs and medical history will be coded according to the latest MedDRA dictionary version. Previous and concomitant medication and vaccines will be coded according to the latest version of the WHO Drug dictionary.

18.4 Investigator File

18.4.1 Maintenance

The Investigator will maintain complete and accurate study documentation in a separate file (i.e. Investigator File) provided during the initiation visit. The Investigator is responsible for maintaining complete, up to date and accurate study records to enable the conduct of the study to be fully documented. The records should include the clinical protocol as well as any amendments, study approval letters, all original ICFs, drug dispensing and accountability logs and all relevant correspondence pertaining to the study.

18.4.2 Archiving and destruction

All study-related documents should be kept by the Investigator for the maximum period of time required by local regulations. No study document should be destroyed without prior written agreement between the Investigator and the Sponsor. Study documents will be stored as per the CIR SOP and will remain at the CIR or the offsite storage facility for the required timeframe. Should the Investigator elect to assign the study documents to another party, or move them to another location, the Sponsor must be notified.

18.4.3 Provision of Additional Information

On request, the Investigator will supply the Sponsor with additional data relating to the study or copies of relevant source records, duly anonymized. In case of particular issues or governmental queries, it is also necessary to have access to the complete study records, provided that the subject's confidentiality is protected in accordance with applicable regulations.

19. PUBLICATION POLICY

All results generated in this study will be considered to be strictly confidential. The Investigator may not submit the results for publication or presentation without prior written permission of the Sponsor. Authorship for any publication will be determined in mutual agreement. Within the scope of publication, co-authorship may be offered, at the sole discretion of the Sponsor, on a case by case basis taking scientific contribution into consideration. This is according to uniform requirements for manuscripts submitted to biomedical journals proposed by the International Committee of Medical Journal Editors.

20. LIABILITIES AND INSURANCE

In case of any damage or injury occurring to a subject in association with the participation in the study, insurance has been contracted.

The name, address and the insurance policy number will be given to the Investigator prior to start of enrollment.

The Investigator is responsible for dispensing the investigational product according to this protocol, and for its secure storage and safe handling throughout the study.

21. STUDY PARTICIPANTS LIST

[redacted]

Clinical Trial Sites	Center for Immunization Research (CIR) Isolation Unit 301 Building 301 Mason Lord Drive Suite 4300 Baltimore, MD 21224 CIR Outpatient Clinic 624 N. Broadway, Hampton House Rm. 117 Baltimore, MD 21205
Site Investigational Product Accountability	Enterics Disease Lab Johns Hopkins University School of Public Heath, Baltimore, MD, 21205 Johns Hopkins Bayview Medical Center Department of Pharmacy Research 4940 Eastern Avenue, AA bldg., Rm 149 Baltimore, MD 21224
Clinical laboratories	[redacted] Quest Diagnostics, Inc. 1901 Sulphur Spring Rd, Baltimore, MD 21227

Johns Hopkins Hospital
600 North Wolfe Street
Baltimore, MD 21287

Johns Hopkins Bayview
4940 Eastern Ave.
Baltimore, MD, 21224

[redacted]

Data Management	The Emmes Corporation 401 N. Washington St. / Suite 700 Rockville, MD 20850
Biostatistics	Assign Data Management & Biostatistics Stadlweg 23 6020 Innsbruck, Austria
Institutional Review Board	JHSPH IRB 615 N. Wolfe Street / Suite E 1100 Baltimore, MD 21205 Telephone: 410-955-3193 Fax: 410-502-0584 Email: JHSPH.irboffice@jhu.edu

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