Clinical Study Protocol

EudraCT No.	2021-001541-13
Investigational Product	ETVAX®; An oral inactivated vaccine against ETEC containing five antigens; four different inactivated *E. coli* strains overexpressing the most prevalent ETEC colonization factors (CFs), i.e. CFA/I, CS3, CS5 and CS6, recombinant protein LCTB*A* and the adjuvant double mutant LT (dmLT)
Study code:	OEV-125
Protocol Version and date	Final 5.0, 23JUNE2022

STUDY TITLE

A Phase 2 immunological bridging study assessing the non-inferiority of a new formulation of ETVAX®. A prospective double-blind, randomized study in healthy volunteers.

Test product and dosage	New partially dried formulation of ETVAX®
Comparator product and dosage	Wet formulation of ETVAX®
Duration of treatment	Two vaccinations, two weeks apart.
Medical Advisor (Sponsor signatory)	[redacted] Scandinavian Biopharma Holding AB, Solna, Sweden [redacted]@scandinavianbiopharma.se
Principal Investigator	[redacted] Clinical Trial Center, Göteborg, Sweden [redacted]

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

Amendment No.	**Date of Amendment**	**Revised protocol version** *(if applicable)*
1.0	16 Jan 2022	Version 3.0, dated 16 Jan 2022
2.0	15 Mar 2022	Version 4.0 dated 15 Mar 2022
3.0	23 June 2022	Version 5.0 dated 23 June 2022

2 PROTOCOL SYNOPSIS

Study Title: A Phase 2 immunological bridging study assessing the non-inferiority of a new formulation of ETVAX®. A prospective double-blind, randomized study in healthy volunteers.	
Study code: OEV-125	**EudraCT No:** 2021-001541-13
Study period Estimated date of first subject enrolled: Q4 2021 Estimated date of last subject completed, main study: Q3 2022 Estimated date of last subject completed, exploratory study: Q4 2022	**Phase of development: 2**
Principal Investigator [redacted], MD Clinical Trial Center (CTC) [redacted] SE-413 46 Göteborg, Sweden	
Study design A prospective double-blind, randomized, parallel-group study with the aim to demonstrate non-inferiority, in terms of immunogenicity, between the wet formulation and a newly developed partially dried formulation of selected components of ETVAX®.	
Objectives Primary objective(s) The aim of this study is to demonstrate non-inferiority, in terms of immunogenicity, between the wet formulation and the newly developed partially dried formulation of selected components of ETVAX®. Secondary objectives To evaluate the safety and tolerability of the new formulation of the vaccine.	

Number of subjects planned

A total number of 126 subjects will be included in each arm of the study, i.e. 252 subjects in total. Assuming a 10% dropout rate the target number of subjects to be recruited per study arm is therefore 140, i.e. 280 subjects in total.

Diagnosis and eligibility criteria

Healthy volunteers fulfilling the following criteria will be eligible for enrolment into the study:

Inclusion criteria

- Male or female aged 18-50 years, inclusive at the time of signing the informed consent.
- Healthy constitution as established by medical history and physical examination.
- Willing and able to give written informed consent for participation in the study.
- Able to comply with study activities, as judged by the Investigator.
- Female Participants: Women of child-bearing potential (for definition see Section 9.3.6):

  - Have to agree to use an acceptable birth control method during participation in the investigation (see Section 9.3.6).

  - A negative pregnancy test (beta human chorionic gonadotropin dipstick test in urine) at Visit 2/Day 1 will be required.
- Male Participants:

  - Have to agree to remain abstinent (refrain from heterosexual intercourse) or use a condom, and agreement to refrain from donating sperm, as defined in Section 9.3.6.

Exclusion criteria

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

- An acute or chronic medical condition that, in the opinion of the investigator/physician, would render ingestion of the investigational products unsafe or would interfere with the evaluation of responses. This includes, but is not limited to gastrointestinal diseases, and autoimmune diseases.
- Current malignancy or history of malignancy during the last five years, based on anamnesis.
- Gastroenteritis within two weeks prior to vaccination.
- Regular use of laxatives, antacids or other agents that lower stomach acidity.
- Any planned major surgery during the duration of the study.
- After 10 minutes supine rest, any vital signs outside the following ranges:

  -Systolic BP > 160 mm Hg
  -Diastolic BP > 100 mm Hg
  -Heart rate < 40 or >85 beats per minute
- Antibiotic therapy within two weeks prior to the vaccination.

- Known Hepatitis A, B, C, and/or HIV infection.
- Concomitant intake of immunomodulating drugs during the study period or less than 3 months prior to the first immunization, with the following exceptions: oral anti-histamines are not allowed during the study period or less than 3 weeks prior to the first immunization. Local anti-histamine treatment is allowed during the study period.
- Any other significant medical conditions (e.g. poorly controlled psychiatric condition) judged by the Investigator to preclude entry.
- Intends to receive any other vaccine during the study period, or within two weeks prior to trial vaccination.
- Has previously received Dukoral or any type of enterotoxigenic *Escherichia coli* (ETEC) or cholera vaccines.
- Brought up in ETEC-endemic areas (e.g., urban and rural areas of Central and South America, Caribbean, most countries in Asia, Africa, etc.).
- Has travelled to ETEC-endemic areas within the last 3 years OR spent > two months in ETEC endemic areas during the last 10 years.
- Intends to travel to ETEC endemic countries during the study period.
- Known or suspected history of drug, chemical or alcohol abuse, as deemed by the investigator/physician.
- History of severe allergy/hypersensitivity or ongoing allergy/hypersensitivity, as judged by the Investigator, or history of hypersensitivity to drugs with a similar chemical structure or class to ETVAX®.
- Participation in any other clinical study that included drug treatment with the last administration within the past 3 months prior to administration of treatment in this study. Patients consented and screened but not dosed in previous clinical studies are not excluded
- Concomitant participation in any other clinical study.
- Females who are pregnant as determined by urine test at inclusion and prior to each vaccination.
- Females who are nursing.
- Unable to participate in all study visits.
- Any condition or circumstance which would make the subject unsuitable for participation in the study in the opinion of the investigator/physician.

Methodology

Consenting subjects will be screened for eligibility, according to study-specific inclusion/exclusion criteria within 30 days prior to the first administration of Investigational Medicinal Product (IMP) (Visit 1; Screening visit). Eligible subjects will be randomized on Day 1 (Visit 2) to receive either of the two oral formulations of ETVAX® (1:1) and consecutively included the study. The treatment allocation (Wet formulation/Partially dried formulation) will be double-blinded, *i.e.* it will not be disclosed to the subjects, site staff, laboratory staff or Sponsor.

Study subjects will receive two oral doses, two weeks apart (Day 1/Visit 2 and Day 15 (±2) /Visit 3).

If a subject is unable to come for their third study visit on day 15±2 due to e.g. illness or rules regarding quarantine due to the COVID-19 epidemic, the second vaccine dose can be administered up to 28 days after the first dose.

Dosing will occur at the clinic. After each dose, subjects will remain at the clinic for observation for 15 min before leaving.

At each dosing visit (Day 1 and Day 15) subjects will receive adverse event and medication diaries to be completed for six days after each vaccination (i.e. day of vaccination and five subsequent days).

A follow-up visit will be performed 7 days (acceptable range: 6-10 days) after the last (second) dose in all study subjects.

Blood samples will be collected on visit 2 and 4.
For the exploratory analyses, subgroups of subjects (n=20-40, evenly distributed between the two treatment arms) will participate in additional follow-up visits 5± 1, 30± 7 and 75-125 days after the second dose. Blood samples will be collected on all exploratory visits. The extra visits and analyses for exploratory analyses may continue after the main part of the study has been completed and the database locked.

Investigational Medicinal Product, dosage and mode of administration

ETVAX® is an oral inactivated vaccine against ETEC containing four different inactivated *E. coli* strains overexpressing the most prevalent ETEC colonization factors (CFs), i.e. CFA/I, CS3, CS5 and CS6, a recombinant protein LCTB*A* and the adjuvant double mutant LT (dmLT).

The product formulation of ETVAX® used so far in clinical trials (wet formulation) is a liquid suspension of inactivated bacteria and LCTB*A* in one vial, freeze-dried dmLT adjuvant in a second vial, and effervescent buffer granules in a separate sachet. Prior to administration, the buffer is dissolved in 150 ml tap water, followed by the addition of the content of the vaccine vial (inactivated bacteria mixed with LCTB*A*) and reconstituted adjuvant dmLT from the second vial.

As a part of manufacturing development process, a method to spray-dry dmLT together with LCTB*A* protein has been established. In the new, partially dried formulation, the dmLT and LCTB*A* are spray-dried and mixed with the buffer granules and stabilizing excipients in a sachet. In preparation of the partially dried formulation, the content of the buffer sachet (buffer, dmLT, and LCTB*A*) is dissolved in 150 ml tap water, followed by the addition of the vaccine vial content (inactivated bacteria). The new product formulation is thus very simple to prepare and suitable for use in the field.

Non-Investigational Medicinal Products, dosage and mode of administration *(if applicable)*

Not applicable in this study.

Duration of treatment

Subjects will receive two oral doses of the vaccine, two weeks apart.

Duration of subjects' involvement in the study

The duration of subject's participation in the study will be between 3 and 9 weeks for the main study, including a 0-4 week screening period, 2-4 week treatment period and 1 week of follow-up.

For subjects who also participate in the exploratory sub-study with follow-up until 75-125 days after dose 2, the total duration will be between 13 and 26 weeks, including follow-up visits on Days 30± 7 and 75-125 after the second (last) dose.

Efficacy assessments

Seroconversion rates of IgA and/or IgG anti-LTB antibodies in serum:
IgA and IgG antibodies specific for LTB will be measured in serum samples collected on Day 1/Visit 2 and Day 7 after the second dose/Visit 4 using an Enzyme Linked Immunosorbent (ELISA) assay. The analyses will be performed by technical laboratory personnel at the Department for Microbiology and Immunology, University of Gothenburg, according to standardized laboratory procedures. The seroconversion rate for IgA and IgG will be determined as the frequencies of subjects having a ≥2-fold increase in LTB-specific antibody levels in post compared to pre-vaccination samples.

Safety assessments

Physical examination

A complete physical examination will include assessments of the head, eyes, ears, nose, throat, skin, thyroid, neurological, lungs, cardiovascular, abdomen (liver and spleen), lymph nodes and extremities.

Vital signs

Systolic and diastolic blood pressure and heart rate will be measured in the supine position after 10 minutes of rest. Body temperature will be measured in the ear using a digital thermometer.

Subject Diary

The study diaries will contain questions regarding adverse events and current medication. The diaries should be filled in by the subjects on the day of vaccination and five subsequent days. Information about adverse events obtained from the diaries will be verified by interviewing the subject and will then be registered in the CRFs by the study nurse/physician.
The following solicited AEs will be included in the diary: fever, nausea, vomiting, abdominal pain and loose stools/diarrhea.

Statistical methods

Assuming a seroconversion rate of 85% in both the wet and the partially dried formulation, and aiming to prove non-inferiority with margin -15% (on an absolute scale), 126 subjects are needed in each treatment arm to achieve 90% power. This calculation was done with Farrington-Manning's method, assuming that the study will be evaluated by a two-sided 95% confidence interval for the difference between the seroconversion rates of the two treatment groups.

A 10% dropout rate is assumed. The target number of subjects to be recruited per study arm is therefore 140**.**

The primary endpoint will be summarized for the Full analysis set (FAS) and the Per protocol analysis set (PPAS). The primary endpoint analysis of PPAS will be considered the primary analysis of the study.

The primary endpoint will also be summarized with descriptive statistics for the PPAS and FAS.

3 TABLE OF CONTENTS

4 LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Abbreviation or term	Explanation
AE	Adverse event
ATC	Anatomic Therapeutic Chemical
CF	Colonization Factor
CI	Confidence Interval
CT	Cholera Toxin
CTC	Clinical Trial Center at Sahlgrenska University Hospital
CS	Coli surface antigen
CRF	Case Report Form
CRO	Clinical Research Organization
CSR	Clinical Study Report
CTA	Clinical Trial Agreement
CV	Curriculum Vitae
DMID	Division of Microbiology and Infectious Diseases
DMP	Data Management Plan
dmLT	Double mutated heat-labile toxin
ECL	Electrochemiluminescence
ELISA	Enzyme Linked Immunosorbent Assay
EPM	Etikprövningsmyndigheten
ETEC	Enterotoxigenic *Escherichia coli*
FAS	Full analysis set
GCP	Good clinical practice
GLP	Good laboratory practice
GMP	Good manufacturing practise
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council for Harmonization
IEC	Independent Ethics Committee
Ig	Immunoglobulin
IMP	Investigational medicinal product

Abbreviation or term	Explanation
ISF	Investigator Site File
iSRC	internal Safety Review Committee
LCTB*A*	Hybrid protein between the B-subunit of the *E. coli* heat-labile enterotoxin (LTB) and the B-subunit of the cholera toxin (CTB)
LMIC	Low and middle income countries
LT	Heat-labile toxin
LV	Läkemedelsverket
MedDRA	Medical Dictionary for Regulatory Activities
NIH	National Institutes of Health
PPAS	Per protocol analysis set
PBMC	Peripheral Blood Mononuclear Cells
PBS	Phosfate Buffer Solution
PT	Preferred term
QP	Qualified Person
RN	Registered Nurse
SAR	Serious Adverse Reaction
SAE	Serious Adverse Event
SBH	Scandinavian Biopharma Holding AB
[redacted]	[redacted]
SDV	Source Data Verification
SIgA	Secretory IgA
SOC	System Organ Class
ST	Heat-stable toxin
SUSAR	Suspected Unexpected Serious Adverse Reaction
TD	Travellers' Diarrhea
WOCBP	Women of childbearing potential
Enrolled subject	Subject who has signed the Informed Consent Form (ICF)
Screen failure	Enrolled subject not included

Abbreviation or term	Explanation
Included subject	Subject randomized
Withdrawn subject	Subject randomized but not completed
Completed subject	Subject completed the study period, including follow-up

5 ETHICS AND REGULATORY REQUIREMENTS

5.1 Ethical conduct of the study

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

The Declaration of Helsinki is included as Appendix 12.2 to the Protocol.

5.2 Ethics and regulatory review

The Principal Investigator is responsible for submission of the Clinical Study Protocol, the Subject Information and Informed Consent Form (ICF), any other written information to be provided to the subjects and any advertisements used for recruitment of study subjects to Etikprövningsmyndigheten (EPM), the applicable Independent Ethics Committee (IEC), for approval.

The Sponsor, Scandinavian Biopharma Holding AB (SBH), is responsible for submission of study documents to Läkemedelverket (LV), the applicable Competent Authority in Sweden.

Approval must be obtained in writing from both Competent Authority (LV) and IEC (EPM) before the first study subject can be enrolled.

SBH will provide the Competent Authority (LV), IEC (EPM) and Principal Investigator with safety updates/reports according to local requirements.

5.3 Subject information and consent

It is the responsibility of the Investigator to give each potential study subject adequate verbal and written information regarding the objectives and the procedures of the study as well as any risks or inconvenience involved before including the subject in the study. The subject must be informed about the right to withdraw from the study at any time. The subject should be allowed sufficient time for consideration of the proposal.

Furthermore, it is the responsibility of the Investigator to obtain signed informed consent from all subjects before including them in the study. The ICF must be signed and personally dated by the subject and by the person who conducted the informed consent discussion before any investigation-specific procedures are performed, including screening procedures. A copy of the Subject Information and the signed ICF will be provided to the subject.

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

The final version of the Subject Information sheet and ICF is submitted to the IEC (EPM) and must not be changed without permission from SBH and EPM.

5.4 Subject Information Card

The subject will be provided with a Subject Information Card. The card will at least include the following information:

- That he/she is participating in a clinical study
- Subject study ID
- That he/she is treated with the Investigational Medicinal Product (IMP)
- The name and phone number of the Investigator
- Name and address of the Sponsor

5.5 Subject data protection

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

The ICF includes information that data will be recorded, collected and processed. In accordance with the European General Data Protection Regulation (2016/679), the data will not identify any persons taking part in the study.

The potential study subject must be informed that by signing the ICF he/she approves that authorized representatives from SBH, the IEC (EPM) and Competent Authority (LV) have direct access to his medical records for verification of clinical study procedures.

The subject has the right to request access to his personal data and the right to request rectification of any data that is not correct and/or complete.

6 INVESTIGATOR(S) AND STUDY ADMINISTRATIVE STRUCTURE

This clinical study is sponsored by the Swedish company Scandinavian Biopharma Holding AB. The study will be conducted at Clinical Trial Center's (CTC's) facilities at Sahlgrenska Hospital in Gothenburg, Sweden.

Immunological analyses will be performed at the [redacted].

Clinical Monitoring, Data Management, biostatistics and SUSAR reporting will be provided by [redacted]. Key members of the Sponsor, CTC, University of Gothenburg and CRO project teams and sub-contractors are presented below.

Sponsor

Scandinavian Biopharma Holding AB

Industrivägen 1
SE-171 48 Solna, Sweden

Sponsor's Signatory

[redacted]

Academic co-investigator:

[redacted]

Clinical Trial Center (CTC)
[redacted]
SE-413 46 Göteborg, Sweden

Principal Investigator

[redacted]

Clinical Monitoring, Pharmacovigilance/SUSAR reporting, Data Management and Statistics

[redacted]

Laboratory [redacted]

Signatures required should be provided in appendix 12.1.

7 INTRODUCTION

7.1 Project background

Enterotoxigenic *Escherichia coli* (ETEC) bacteria cause considerable physical suffering and malnutrition due to repeated bouts of illness in children in low- and middle-income countries (LMIC). Diarrhoea is the second-leading cause of death among children <5 years of age worldwide. The actual mortality numbers are difficult to estimate; global annual incidences of nearly 1.7 billion cases of childhood diarrhoeal disease with approximately 500 000 – 700 000 deaths in children <5 years of age have been presented [1, 2]. The relative importance of ETEC versus other diarrhoeal pathogens for the total burden of diarrhoeal disease is debated [2, 3]. However, the significant morbidity caused by ETEC in young children is undisputed, and it is also clear that repeated ETEC episodes have significant impact on growth and mental development [4-6]. WHO's PDVAC (Product Development for Vaccines committee) has 2020 expressed that an ETEC vaccine is a high priority for LMIC [4, 5]. A vaccine against ETEC would have a significant impact on travelers as well. Approx. 80 million travelers visit subtropical/tropical countries annually [7]. Traveling to LMIC involves a risk of travel-related diseases and at least 35 million travelers per year are affected by travelers' diarrhea (TD) [8, 9]. ETEC is the major cause of TD, responsible for approximately 15 million annual cases [8, 9].

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

heat stable toxin (ST) triggering diarrhoea. The LT toxin is highly homologous to the cholera toxin (CT) and cause diarrhea via a highly similar mechanisms [6].

ETVAX® is an oral inactivated vaccine against ETEC containing four different inactivated *E. coli* strains overexpressing the most prevalent ETEC CFs, i.e. CFA/I, CS3, CS5 and CS6, a recombinant protein LCTB*A* and the adjuvant double mutant LT (dmLT) [6, 10]. ETVAX® is currently in late phase development and has been studied in several clinical phase 1 and 2b studies. Successfully completed clinical studies have shown that the vaccine is safe and immunogenic, giving rise to mucosal IgA responses to all vaccine CFs and to LTB, in children living in endemic areas as well as in Western healthy adults [11-15]. Our previous results also demonstrate that the vaccine gives rise to memory B cell responses that can be detected at least until 2 years after vaccination and that such long-term immunity may be reflected by the circulation of activated follicular helper T cells early after immunization [13, 16].

The product formulation of ETVAX® used so far in clinical trials is a liquid suspension of inactivated bacteria and LCTB*A* in one vial, freeze-dried dmLT adjuvant in a second vial, and effervescent buffer granules in a separate sachet. Prior to administration, the buffer is dissolved in 150 ml tap water, followed by the addition of the content of the vaccine vial (inactivated bacteria mixed with LCTB*A*) and reconstituted and diluted adjuvant dmLT from the second vial. As a part of manufacturing development process, a method to spray-dry dmLT together with LCTB*A* protein has been established. In the new, partially dried formulation, the spray-dried dmLT and LCTB*A* are mixed with the buffer granules and stabilizing excipients in a sachet. In preparation of the partially dried formulation the content of the buffer sachet (buffer, dmLT, and LCTB*A*) are dissolved in 150 ml tap water, followed by the addition of the vaccine vial content (inactivated bacteria). The new product formulation is thus very simple to prepare and suitable for use in the field.

A two-armed, non-inferiority, immunogenicity and safety study comparing the two formulations will be conducted in 280 adult volunteers at Clinical Trial Center (CTC) located at the Sahlgrenska University Hospital in Gothenburg, Sweden.

Due to difficulties to get a reliable measurement of immune responses to the CFs in serum of non-primed (i.e. previously not immunized nor infected) individuals, only the immune response rates induced by the LCTB*A* part of the vaccine to the binding subunit of LT (LTB) will be measured. For oral vaccines, the responses are evoked in the gut and only some immune responses, e.g. against LTB for ETVAX®, are detectable in serum of immunised individuals [6, 12].

7.2 Investigational Medicinal Product (IMP)

7.2.1 Vaccine development

Protection against ETEC is most likely mainly provided by antibodies against the different CFs and LT produced locally in the gut. Studies in experimental animals and human subjects strongly support that the CFs are key protective antigens against ETEC expressing homologous CFs. Thus, studies in a birth cohort in Bangladesh have shown that re-infections with ETEC expressing homologous CFs are very rare, at variance with re-infections with ETEC expressing heterologous CFs [17], supporting the importance of CF immunity in an ETEC vaccine. The current ETVAX® formulation includes four of the most prevalent CFs, i.e. CFA/I, CS3, CS5, CS6.

Clinical trials both in travellers and in endemic countries have also shown that cholera toxin B subunit (CTB), which is highly homologous to LTB, may provide significant protection against disease caused by LT expressing ETEC. However, it has been suggested that an LT-like toxoid may provide stronger protection against LT ETEC, at least in young, immunologically naïve children in comparison to CTB. Thus, it has been previously shown that human subjects challenged with LT producing ETEC as well as Bangladeshi patients convalescing from *E. coli* LT diarrhoea responded with significantly stronger local and serum antibody responses to LT than to CT [18, 19]. ETVAX® includes LCTB*A*, a hybrid between LTB and CTB [20], which has been shown to induce strong neutralizing LTB-specific antibody responses in both serum and mucosal samples in several preclinical and clinical studies [10-12, 14, 15].

Since ETEC infections are confined to the mucosal surfaces in the gut, immune protection is most likely predominantly provided by locally produced secretory IgA (SIgA) antibodies against major protective antigens, i.e., CF and LT antigens [6]. However, strong LTB-specific IgA and IgG responses can also be detected in serum after vaccination with ETVAX® including LCTB*A,* and these responses correlate significantly with LTB-specific IgA responses measured in samples reflecting mucosal immunity, such as secretions from antibody secreting cells migrating to the intestinal mucosa [11, 12, 14]. Serum from immunized subjects also have strong LT toxin neutralizing capacity, and the functional antibody activity correlate well with antibody titers measured by ELISA [12]. Levels of LTB-specific antibodies measured in serum after ETVAX® vaccination thus reflect both mucosal immune responses and antibody function.

7.2.2 Mucosal Adjuvants

The LT toxin has been shown to have inherent mucosal adjuvant properties for co-administered antigens and thus has potential as a mucosal adjuvant for different co-administered vaccines [21]. Several animal and human studies have evaluated LT as a mucosal adjuvant for co-administration with inactivated whole cell or purified subunit antigens derived from a wide range of bacterial and viral pathogens [21]. Despite the significant potential of LT as a mucosal adjuvant, oral LT has considerable enterotoxicity for humans, thereby limiting its use as an adjuvant [21, 22]. Therefore, efforts have been made to construct LT molecules with decreased enterotoxicity, but with undiminished adjuvant properties. As described in more detail below, single mutant LT (mLT) was developed by Dr. John Clements and colleagues at Tulane University [23], but a high oral dose (100 µg) was still found to be reactogenic when given alone. Also, despite impressive adjuvant activity, mLT retained some level of gastrointestinal reactogenicity in Phase I trials when combined

with bacterial vaccine antigens [24]. Subsequently, double mutant LT (dmLT) was developed by the investigative team at Tulane as a 2nd generation mucosal adjuvant with further reduced enterotoxicity [21, 25]; dmLT has been proven to be safe as a stand-alone vaccine in a phase I single dose escalation study (Protocol 13-0013; clinicaltrials.gov NCT02531685) [26], and together with an attenuated ETEC vaccine in a 25 µg/dose phase 2b study (clinicaltrials.gov, NCT01060748)[27].

7.2.3 Development and testing of double mutant LT (dmLT)

E. coli JM83(pLC403) codes for production of the genetically modified form of *E. coli* LT in which the arginine at amino acid position 192 has been replaced with glycine and the leucine at amino acid position 211 has been replaced with alanine in the A subunit of LT [25]. These two amino acid substitutions are essential in known and putative proteolytic cleavage sites in the LT protein that are considered to be critical for activation of the secreted toxin molecules. The protein coded by this strain was designated LT(R192G/L211A), i.e. dmLT, and has been extensively evaluated for residual enterotoxicity *in vitro* and *in vivo* studies, and in pre-clinical animal studies for its ability to function as an adjuvant for co-administered antigens [21, 25]. Two oral doses of dmLT in rats were also found to lack local and systemic toxicity in a GLP toxicology study conducted by PATH and Division of Microbiology and Infectious Diseases (DMID), National Institutes of Health (NIH) [28]. [redacted]

[redacted]

[redacted]

7.2.4 Non-clinical safety

7.2.4.1 Immunogenicity and safety in mice of ETEC vaccine with and without dmLT

ETEC vaccine given alone or together with the dmLT adjuvant was studied in mice after oral immunizations for its capacity to induce intestinal and serum antibody responses to the different vaccine components. Small intestine or fecal IgA responses showed 2 to 50-fold increase in specific antibodies to the different vaccine components following immunization [10]. Immune responses were further increased when the vaccine was given in combination with the oral adjuvant [redacted].

A significant IgA immune response was also obtained in serum to each of the vaccine components and this was even further pronounced for IgG + IgM type antibodies. In most cases, the serum immune responses were further enhanced in the presence of the adjuvant [10]. In addition to demonstrating immune enhancement, this study also showed that when adding different dosages of dmLT when administered with vaccine, the adjuvant effect of dmLT was especially strong for lower vaccine dosages [10]. This suggests the potential for vaccine dose sparing when an effective adjuvant is added, which could be especially valuable for use with ETVAX® in younger age groups in which fractional doses of vaccine may be needed [29].

[redacted]

7.2.4.2 cGLP Toxicology and Immunology Evaluation of ETEC vaccine with and without dmLT

A repeated dose cGLP toxicity study was conducted in C57BL/6J mice, in which the main objective was to investigate whether ETEC vaccine produced toxic reactions after repeated per-oral administration in the animals. ETEC vaccine was administered in two different doses, 5 x the human clinical dose and 25 x the human clinical dose, and administered with or without the addition of the dmLT adjuvant (lot 1575). Phosphate buffer solution (PBS) was used as the control item.

In comparison to PBS treated animals the vaccines in high doses affected the general health slightly but no major pathological, clinical chemistry or haematological observations were found. Acute gastritis was seen in some animals that were most probably the results of previous and passing injury on a microscopic level. The gastritis was deemed to be transient and did not raise any safety concerns by the Swedish Medical Product Agency. Furthermore, symptoms consistent with acute gastritis have not been observed in any of the Phase I/II trials in which dmLT was co-administered with ETEC vaccine or when combined with a live-attenuated ETEC vaccine in doses of up to 25 µg. A strong anti-LCTB*A* response in serum was detected across all dosing groups in the mouse cGLP study while the serum anti-CFA/I response was somewhat weaker and dependent on the dosing group.

In conclusion, the vaccine was shown to be safe and immunogenic when tested in mice; adjuvantation with dmLT increased the immune response without altering the safety profile.

7.2.5 Clinical experience

7.2.5.1 Vaccine safety

ETVAX® has been shown to have an excellent safety profile. In the OEV-121 study (Double-blind, Randomised, Placebo Controlled Phase I study to Evaluate the Safety and Immunogenicity of an Oral inactivated Tetravalent ETEC Vaccine Alone and in Combination with dmLT Adjuvant in Healthy Adult Volunteers, EudraCT number 2011-003228-11) in adult Swedish volunteers, administration of two oral doses of ETEC vaccine was shown to be safe irrespective of given alone or in combination with either 10 or 25 µg dmLT, respectively, with no differences in adverse events (AEs) in vaccinees and placebo recipients [12]. In the OEV-121A study, a single dose of ETEC vaccine alone, without adjuvant, was administered to 57 subjects that had received either two doses of ETEC vaccine alone or ETEC vaccine +10 µg dmLT 1-2 years previously or had never been vaccinated against ETEC before (A Phase I Study to Evaluate the Capacity of an Oral Inactivated Tetravalent ETEC Vaccine

Alone and in Combination with dmLT Adjuvant to Induce Immunological Memory to Toxin and Colonization Factor Antigens in the Vaccine, EudraCT number 2013-003693-28) [13]. Few AEs were recorded in the subjects participating in the study and there were no significant differences between subjects receiving a first or third vaccine dose.

ETVAX® safety was also documented in a recent age-descending study in Bangladesh (A Randomised, Double- blind, Placebo-controlled, Dose-Escalation Study Evaluating the Safety, Tolerability, and Immunogenicity of an Oral Inactivated ETEC Vaccine Alone and Together With dmLT Adjuvant in age-descending groups in Bangladesh, ClinicalTrials.gov number NCT02531802) encompassing 495 subjects (OEV-122 study) [14, 15]. In this study, immunisations started with full vaccine dose with or without 10 µg dmLT in adults, followed by administration of different fractional vaccine and dmLT doses in children 2-5 years, 1-2 years and finally infants aged 6-12 months. In all age groups, the highest safe dose was identified and then given with different doses of dmLT. There was no progression to younger age groups before safety was assessed in the older groups. The vaccine was safe in the adults with no significant differences in AEs between vaccinees and placebo recipients [14].

In the children, no clear safety signals were identified although vomiting occurred in children when higher doses were tested [15]. However, vomiting was decreasingly observed with decreasing dose and increasing age, similar to what had been observed in studies with a first-generation whole cell inactivated ETEC vaccine [30]. Furthermore, dmLT did not appear to contribute to tolerability issues. When vomiting occurred, the reaction was transient, self-limited and generally mild, and mitigated by selection of lower doses, as was the objective of the dose escalation phases (i.e. identification of highest tolerated dose) [15].

A phase 2b study of Finnish adult travellers to Benin (A randomized, placebo-controlled phase IIb (OEV-123) study to evaluate safety, immunogenicity, diagnostic methodology, and estimate vaccine efficacy of an oral enterotoxigenic *Escherichia coli* (ETEC) Vaccine (ETVAX®) for prevention of clinically significant ETEC diarrhea in healthy adult travelers visiting West Africa, EudraCT No.: 2016-002690-35) encompassing 749 randomized subjects (ITT set) whereof 374 received vaccine and 375 received placebo was recently finalized. In this study the vaccine was shown to have an excellent safety profile and no statistical difference regarding safety was found between vaccine and placebo recipients.

7.2.5.2 Vaccine immunogenicity

The immunogenicity of ETVAX® has previously been documented in Swedish adults in the OEV-121 study. The results showed that a full adult dose of ETEC vaccine administered together with 10 µg dmLT adjuvant resulted in 93% IgA and/or IgG seroconversion (≥2-fold increase) against LTB in samples collected 7 days after the second dose [12]. The vaccine also induced strong IgA responses against both LTB and CFs in fecal samples and antibodies in lymphocyte supernatants (ALS) specimens, reflecting mucosal immune responses. Serum from immunized subjects also had strong LT toxin neutralization activity [12]. Serum

antibody responses against LTB also correlated significantly with LTB-specific mucosal responses measured in ALS specimens. dmLT adjuvant significantly enhanced ALS IgA responses to CS6, which is the antigen present in the lowest amount in the ETVAX vaccine, supporting the dose-sparing effect of dmLT previously observed in mice [10, 12].

In the OEV-122 study 100% of the adult Bangladeshi subjects receiving the vaccine alone or with 10 µg dmLT responded with an at last 2-fold response in anti-LTB IgA antibodies in both serum and ALS specimens [14]. ALS IgA responses to CFs were also observed in 100% of subjects. The high response is probably reflecting that in Bangladesh a high percentage of the population has experienced ETEC infections prior to vaccination.

Mucosal (ALS) as well as plasma IgA responses in Bangladeshi children 2-5 years were comparable to those in adults both in magnitude and frequencies [15]. Responses in children 1-2 years were also good with ALS IgA responses to all five antigens in a majority of vaccinees and somewhat lower frequencies in plasma. With regard to immune responses in the 6-11 months old infants immune responses to the CFs were lower than in the older age groups. Furthermore, response rates in the infant placebo recipients were considerably higher than in older children, probably due to very high frequencies of asymptomatic ETEC infections in this age group as previously shown in a birth cohort study in Bangladesh [17]. In spite of this, mucosal immune responses (ALS and/or fecal SIgA) were significantly higher against all vaccine antigens in the infant vaccinees than in the placebo recipients [15].

In the OEV-123 study 81% of the Finnish vaccine recipients responded with at least a 2-fold increase in anti-LTB IgA serum antibodies after vaccination with ETEC vaccine + 10 µg dmLT.

7.3 Risk/benefit assessment

7.3.1 Benefits

There are no major benefits for subjects participating in the study. However, subjects will receive a free health check-up and immunization may provide protection against some of the most common types of ETEC and against diarrhoea caused by *Vibrio cholerae* bacteria, which can be beneficial during travel to ETEC and cholera endemic countries.

7.3.2 Risks

Based on experiences from previous studies with ETVAX®, immunization is expected to give rise to only a low frequency of mild and transient symptoms, including a few loose stools, mild nausea/vomiting, and abdominal pain or bloating. In the previous trial including 129 Swedish adults there were no significant differences in either frequency or severity of AEs between the study groups receiving ETEC vaccine with or without dmLT and the placebo group receiving bicarbonate buffer alone [12]. The excellent safety profile of the vaccine was supported by very few recorded AEs in Bangladeshi adults vaccinated with ETEC vaccine

with or without 10 µg dmLT, with no significant differences between subjects in the vaccine (n=30) or placebo groups (n=15) [14].In the recent clinical trial in Finland (OEV-123), no significant differences in AEs were recorded between subjects receiving ETVAX® (n=374) and placebo recipients (n=375).

Since the new partially dried vaccine formulation contains the same vaccine components as the previously tested wet vaccine formulation, the risk of inducing different vaccine associated AEs in subjects receiving the new formulation is deemed to be low.

Blood sampling of study participants may cause some pain at the time of sample collection and may result in the development of a bruise but has no other risks.

The risk for individuals participating in this study is therefore assessed to be very small.

7.3.2.1 Risk assessment for the OEV-125 trial in relation to the COVID-19 epidemic

The risk that the COVID-19 epidemic will influence the possibility to perform the trial according to plan, or influence the results of the trial, is continuously assessed by the study team. Since the majority of the subjects are planned to be enrolled in Q4 2021-Q2 2022, when a majority of the adult Swedish population is predicted to have been vaccinated against COVID-19, the risk that the epidemic will have major influence on the study is deemed to be relatively small.

However, the epidemic situation and changes in the national and local COVID-19 vaccination schedule will be closely monitored.
Some COVID-19 related mitigation measures have already been implemented into the Study Protocol, e.g allowing a longer span between the dosing visits under certain circumstances, see section 9 for details. Detailed information about COVID-19 infections and vaccinations is also collected from all participants, primarily to support evaluation of exploratory endpoints, which include comparisons of immune responses to ETEC and other infections and vaccinations, including COVID-19. In addition, a risk assessment, including mitigations, taking into account the local COVID-19 situation and predictions provided by the authorities will be performed and updated within 60 days of trial initiation. This assessment will cover, but should not be limited to, the following aspects; participant and site staff safety, IMP and laboratory material logistics as well as monitoring. A separate Monitoring plan will also be provided, in which monitoring details will be further described.
The risk-assessment will be documented in the Trial Master File (TMF) and will be updated if the situation changes after study start.

8 STUDY OBJECTIVES AND ENDPOINTS

8.1 Primary objective

The aim of this study is to demonstrate non-inferiority, in terms of immunogenicity, between the “wet formulation” and the newly developed partially dried formulation of selected

components of ETVAX®.

8.1.1 Primary endpoint(s)

The primary endpoint to be measured for each patient in the study is **response (yes/no)** to a vaccine. A vaccine responder will be defined by a ≥2-fold increase in IgA and/or IgG antibody levels against LTB in serum between post- compared to pre-immunization samples. The response rates (seroconversion rates) of IgA and/or IgG anti-LTB antibodies in serum will be derived and compared between the two treatment groups.

8.2 Secondary objectives

To evaluate the safety and tolerability of the new formulation of the vaccine.

8.2.1 Secondary endpoints

Occurrence of solicited symptoms for six days after each vaccination (day of vaccination and five subsequent days).

8.3 Exploratory Objective(s)

To evaluate if ETVAX® vaccination induces circulating antigen specific memory B- and/or T cells that can be assessed using recently established laboratory assays.

8.3.1 Exploratory Endpoint(s)

Levels of IgA and IgG antibodies specific for LTB and CFs in cultures of peripheral blood mononuclear cells (PBMCs) isolated before and after vaccination stimulated with substances that activate memory B cells and transform them into antibody secreting plasmablasts.

Frequencies of CD4+ T helper cells expressing activation markers and/or cytokines in blood samples collected before and after vaccination.

Levels of cytokines in culture supernatants of samples collected before and after vaccination.

9 INVESTIGATIONAL PLAN

This is a prospective double-blind, randomized, parallel-group study with the aim to demonstrate non-inferiority, in terms of immunogenicity, between the wet formulation and a newly developed partially dried formulation of selected components of ETVAX®.

The study will be conducted at one site; CTC in Gothenburg, Sweden.

A total of 280 healthy volunteers is planned to be randomized into the study.
Assuming a 10% drop-out rate, we expect 252 healthy volunteers to complete the study.

Overall description of study design

Consenting subjects will be screened for eligibility, according to study-specific inclusion/exclusion criteria within 30 days prior to the first administration of IMP (Visit 1; Screening visit). Eligible subjects will be randomized on Day 1 (Visit 2) to receive either of the two oral formulations of ETVAX® (1:1) and consecutively included the study.

Screening visit and Day 1 (Visit 2) may be performed on the same day, provided that the Study protocol is adhered to in all aspects, including but not limited to the Subject Information and Consent procedures outlined in section 5.3.

The treatment allocation (Wet formulation/Partially dried formulation) will be double-blinded, *i.e.* it will not be disclosed to the subjects, site staff, laboratory staff or Sponsor.

Study subjects will receive two oral doses, two weeks apart (Day 1/Visit 2 and Day 15 /Visit 3). Preferably, subjects should receive their second dose on Day 15 (±2). However, if a subject is unable to come for the visit on day 15±2 due to *e.g.* illness or rules regarding quarantine due to the COVID-19 epidemic, the second vaccine dose can be administered up to 28 days after the first dose.
Dosing will occur at the clinic. After each dose, subjects will remain at the clinic for observation for 15 min before leaving.
At each dosing visit (Day 1 and Day 15) subjects will receive adverse event and medication diaries to be completed for six days after each vaccination (i.e. day of vaccination and five subsequent days).

A follow-up visit will be performed 7 days (acceptable range: 6-10 days) after the last (second) dose in all study subjects (Visit 4).

Blood samples will be collected on visit 2 and 4.

For the exploratory analyses, subgroups of subjects in the main study (n=20-40, evenly distributed between the two treatment arms) will participate in extra follow-up visits 5± 1 (Visit 3b, n=40), 30± 7 (Visit 4b, n=20) and 75-125 (Visit 4c, n=20) days after the second

dose in addition to the regular visits (Visit 1-4) in the main study. Blood samples will be collected on all exploratory visits. The extra visits and analyses for exploratory analyses may continue after the main part of the study has been completed and the database locked.

For subjects participating in main study, the total volume of blood drawn will not exceed 40 ml. For subjects also participating in the exploratory analyses, the total volume will not exceed 250 ml.

The timing and frequency of study visits and assessments in each study part are presented in the schedules of events in Table 9.1 and 9.2.

Table 9.1 Schedule of events – Main Study assessments

	Visit 1 Screening	**Visit 2 Dose 1**	**Visit 3 Dose 2**	**Visit 4 Follow-up**
Day/assessments	**Day -30 to Day 1[a]**	**Day 1[a]**	**Day 15[b] (±2 days)**	**7 days (6-10 days) after Dose 2**
Informed consent	X			
Demographics and relevant medical/surgical history	X			
Inclusion/exclusion criteria	X	X	X	
Physical examination	X			X
Vital signs (Blood pressure and Heart rate)	X			X
Vital signs (Body temperature)	X	X	X	X
Pregnancy Test (Urine)	X	X	X	
Randomization		X		
Dosing		X	X	
Blood sampling for immunology (primary endpoint)		X		X
Subject diary distribution		X	X	
Subject diary collection			X	X
Baseline events	X[c]	X[c]		
Adverse Event and Serious Adverse Event reporting		X	X	X
Prior and concomitant medications	X	X	X	X

[a]Screening visit and Day 1 (Visit 2) may be performed on the same day.

[b]If a subject is unable to come for the visit on day 15±2 due to *e.g.* illness or rules regarding quarantine due to the COVID-19 epidemic, the second vaccine dose can be administered up to 28 days after the first dose.

[c]Until subject receives IMP

Table 9.2 Schedule of events – Main and Exploratory Study assessments

	Visit 1 Screening	**Visit 2 Dose 1**	**Visit 3 Dose 2**	***Visit 3b Exploratory***	**Visit 4[c] Follow-up**	***Visit 4b Exploratory***	***Visit 4c Exploratory***
Day/assessments	**Day -30 to Day 1[a]**	**Day 1[a]**	**Day 15[b] (±2 days)**	***5 ±1 day after Dose 2***	**7 day (6-10 days) after Dose 2**	***30 ±7 days after Dose 2***	***75-125 days after Dose 2***
Informed consent	X						
Demographics and relevant medical/surgical history	X						
Inclusion/exclusion criteria	X	X	X				
Physical examination	X				X		
Vital signs (Blood pressure and Heart rate)	X				X		
Vital signs (Body temperature)	X	X	X		X		
Pregnancy Test (Urine)	X	X	X				
Randomization		X					
Dosing		X	X				
Blood sampling for immunology (primary endpoint)		X			X		
Blood sampling for exploratory immunological analyses		X[d]		X[d]	X[d]	X[e]	X[e]
Subject diary distribution		X	X				
Subject diary collection			X		X		
Baseline events	X[f]	X[f]					
Adverse Event and Serious Adverse Event reporting		X	X		X		
Prior and concomitant medications	X	X	X		X		

[a]Screening visit and Day 1 (Visit 2) may be performed on the same day.

[b]If a subject is unable to come for the visit on day 15±2 due to *e.g.* illness or rules regarding quarantine due to the COVID-19 epidemic, the second vaccine dose can be administered up to 28 days after the first dose.

[c]Visit 4 should be performed on a separate day compared to Visit 3b.
E.g. if Visit 3b is performed on Day 6 after the second dose, then Visit 4 should be performed on Day 7-10.

[d]Exploratory analyses at Visits 2, 3b and 4 will be performed in 40 subjects.

[e]Exploratory analyses at Visits 4b and 4c will be performed in 20 subjects.

[f]Until subject receives IMP

9.1.1 Dose levels

There is only one dose level in this study; full dose (1/1) of ETVAX®; a tetravalent ETEC vaccine including LCTB*A* and 10 µg double mutant LT (dmLT) and effervescent powder for oral solution.

9.1.2 Stopping rules/Discontinuation Criteria

Scandinavian Biopharma AB has the right to close the study at any time, although this should occur only after consulting involved parties. The IEC (EPM) and the Competent Authority (LV) must be informed. Events that may trigger premature termination of the study include, but are not limited to: new toxicity finding, results of any interim analysis, completed accrual and follow-up of participants, non-compliance with the protocol, change in development plans for the study vaccine, slow recruitment and poor quality data.

9.1.3 Internal Safety Review Committee (iSRC)

There will be no iSRC or Data Monitoring Committee for this study

9.2 Rationale for study design, doses and control group

The new partially dried vaccine formulation is very simple to prepare and suitable for use in the field. In contrast, the old wet vaccine formulation needs to be prepared in several different steps, by trained nurses or pharmacists, and cannot be used in large Phase 3 studies. To ensure that the new partially dried formulation, which includes LCTB*A* toxoid and dmLT in the buffer powder, instead of addition of diluted dmLT from a lyophilized preparation to the vaccine, is not inferior to the old wet formulation of the vaccine, a two-armed, non-inferiority, immunogenicity and safety study comparing the two formulations will be performed in healthy adult Swedish volunteers. This study is important in order to ensure that the new vaccine formulation has a comparable immunogenicity profile as the old vaccine, before evaluating the new vaccine formulation in large Phase 3 studies in ETEC endemic areas.

The study will include healthy Swedish volunteers 18-50 years of age and the inclusion and exclusion criteria will be very similar to previous studies performed at the same study site [11, 12, 31], in order to generate immunogenicity and safety results that are comparable previous studies. The travel history of subjects will be recorded, and subjects likely to have been exposed to ETEC infection during travel will be excluded based on the criteria used in previous studies, in order to minimize variability in in immune responses as a result of on natural immunological priming.

Since the LCTB*A* toxoid is formulated in a new way in the new vaccine, it is particularly important to confirm the immunogenicity of this component. For oral vaccines, the responses are

evoked in the gut and only some immune responses, e.g. against LTB for ETVAX®, are detectable in serum of immunised individuals [6, 12]. Due to difficulties to get a reliable measurement of immune responses to the CF antigens in serum of non-primed (i.e. previously not immunized nor infected) individuals, only the immune response rates raised by the LCTB*A* part of the vaccine to LTB will be measured in this study. Previous studies have shown that serum antibodies induced by ETVAX® have strong LT toxin neutralization activity [12]. Furthermore, the serum responses correlate significantly with LT-specific IgA responses measured in samples reflecting mucosal immunity, such as secretions from antibody secreting cells migrating to the intestinal mucosa [12]. Thus, measurement of serum antibody responses to LTB is a good proxy measurement of toxin neutralizing mucosal antibody responses. LTB-specific antibody responses will be evaluated using assays employed in previous ETEC vaccine trials at the same laboratory (OEV-120, OEV-121 and OEV-121A CSR [11-13]), using a 2-fold increase in antibody responses over baseline as primary endpoint, consistent with the general praxis in the ETEC and mucosal vaccine field.

We have developed a mouse assay to show the adjuvanting capacity of dmLT for release and stability studies (K2038) as well as studied the vaccine with the addition of dmLT in a mouse assay [10]. However, since the spray-dried intermediate and, to an even greater extent, the final formulation contains excipients, buffer components and stabilizers, which makes both the intermediate and the product insoluble at the concentrations and volumes that are required for dosing mice by gastric gavage, a non-inferiority study of the new vaccine formulation in mice is not feasible.

The vaccine will be administered orally twice to each subject at a target interval of 14 days. If the subject is unable to come for the third study visit on day 15 due to eg illness or rules regarding quarantine due to the COVID-19 epidemic, the second vaccine dose can be administered up to 28 days after the first dose. A prolonged interval between vaccine doses did not affect the serum responses to CTB component of the cholera vaccine Dukoral, which is highly homologous to LCTB*A*, when tested in Swedish volunteers [32]. Thus, serum CTB-specific IgA and IgA responses were comparable in both magnitude and frequency in subjects receiving Dukoral 14 days or 28-48 days apart, motivating the extended interval between doses in this ETEC vaccine trial compared to previous trials [32].

Many non-inferiority studies of parenteral vaccines use a 10% non-inferiority margin, but larger margins are also relatively common [33]. The 15% non-inferiority margin used in this non-inferiority study is motivated by the higher individual variability in vaccine responses and lower responder frequencies often observed with mucosal compared to parenteral vaccines. As an example, a comparison an oral inactivated *Shigella flexneri* 2 a vaccine gave a maximum serum response of 70% [34] as compared to a *S. flexneri* 2a conjugate given parentally, where 92-100% of the vaccinees responded in serum [35]. The response rate for the whole cell components of ETVAX® in serum is quite low (3-19% in a Western adult population respond after two doses of vaccine), whereas the mucosal immune responses are much stronger (71-97%) [12]. The response against the LCTB*A* protein is quite different resulting in a 79-90% seroconversion after two doses (OEV-123 CSR, [12]). Still, this is substantially lower than what is seen with many parenteral vaccines. Since no formal correlate of protection exists for ETEC or most other mucosal vaccines, the protective efficacy of the new formulation of the ETVAX® vaccine can only be supported by data from efficacy studies. If the new vaccine is found to be non-inferior to

the old vaccine in this study, this will support that it is reasonable both from an ethical and economical perspective to proceed to test the efficacy of this vaccine formulation in large Phase 3 trials in ETEC endemic areas.

9.3 Selection of study population

9.3.1 Recruitment

The clinical part of the recruitment will be conducted by Clinical Trial Center (CTC) located at the Sahlgrenska University Hospital in Gothenburg, Sweden. This centre has helped recruiting the same category of volunteers, as will be recruited for the non-inferiority bridging study, for all previous studies of ETVAX® in Swedish adult volunteers [11, 12, 31].
Previous experience indicate that the number of recruited subjects should not be a problem, and the historical drop-out rate has been low (less than 5%) [12]. The compliance is also expected to be excellent, based on previous experience. However, due to the ongoing COVID-19 epidemic, a higher drop-out rate is expected (estimation 10%), since even mild symptoms may prevent study visits (See section 9.4.3)

9.3.2 Number of subjects

A total number of 126 subjects needs to be included in each arm of the study in order to achieve 90% power to assess the primary objective. Assuming a 10% dropout rate the target number of subjects to be recruited per study arm is therefore 140.

9.3.3 Screening and enrolment log

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

Subjects who have signed the ICF will be assigned a screening number. Subjects included will be assigned a subject number.

9.3.4 Inclusion criteria

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

1. Male or female aged 18-50 years, inclusive at the time of signing the informed consent.
2. Healthy constitution as established by medical history and physical examination.
3. Willing and able to give written informed consent for participation in the study.
4. Able to comply with study activities, as judged by the Investigator.
5. Female Participants:
    - Women of child-bearing potential (for definition see Section 9.3.6):
        - Have to agree to use an acceptable birth control method during participation in the investigation (see Section 9.3.6).

- A negative pregnancy test (beta human chorionic gonadotropin dipstick test in urine) at Visit 2/Day 1 will be required.

6. Male Participants:

   - Have to agree to remain abstinent (refrain from heterosexual intercourse) or use a condom, and agreement to refrain from donating sperm, as defined in Section 9.3.6

In order to receive Dose 2, subjects must fulfil all the criteria above.

9.3.5 Exclusion criteria

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

1. An acute or chronic medical condition that, in the opinion of the investigator/physician, would render ingestion of the investigational products unsafe or would interfere with the evaluation of responses. This includes, but is not limited to gastrointestinal diseases, and autoimmune diseases.
2. Current malignancy or history of malignancy during the last five years, based on anamnesis.
3. Gastroenteritis within two weeks prior to vaccination.
4. Regular use of laxatives, antacids or other agents that lower stomach acidity.
5. Any planned major surgery during the duration of the study.
6. After 10 minutes supine rest, any vital signs outside the following ranges:
   - Systolic BP > 160 mm Hg
   - Diastolic BP > 100 mm Hg
   - Heart rate < 40 or >85 beats per minute
7. Antibiotic therapy within two weeks prior to the vaccination.
8. Known Hepatitis A, B, C, and/or HIV infection.
9. Concomitant intake of immunomodulating drugs during the study period or less than 3 months prior to the first immunization,with the following exceptions: oral anti-histamines are not allowed during the study period or less than 3 weeks prior to the first immunization. Local anti-histamine treatment is allowed during the study period.
10. Any other significant medical conditions (e.g. poorly controlled psychiatric condition) judged by the Investigator to preclude entry.
11. Intends to receive any other vaccine during the study period, or within two weeks prior to trial vaccination.
12. Has previously received Dukoral or any type of ETEC or cholera vaccines.
13. Brought up in ETEC-endemic areas (e.g., urban and rural areas of Central and South America, Caribbean, most countries in Asia, Africa, etc.).
14. Has travelled to ETEC-endemic areas within the last 3 years OR spent > two months in ETEC endemic areas during the last 10 years.
15. Intends to travel to ETEC endemic countries during the study period.
16. Known or suspected history of drug, chemical or alcohol abuse, as deemed by the investigator/physician.

17. History of severe allergy/hypersensitivity or ongoing allergy/hypersensitivity, as judged by the Investigator, or history of hypersensitivity to drugs with a similar chemical structure or class to ETVAX®.
18. Participation in any other clinical study that included drug treatment with the last administration within the past 3 months prior to administration of study treatment in this study. Patients consented and screened but not dosed in previous clinical studies are not excluded.
19. Concomitant participation in any other clinical study.
20. Females who are pregnant as determined by urine test at inclusion and prior to each vaccination.
21. Females who are nursing.
22. Unable to participate in all study visits.
23. Any condition or circumstance which would make the subject unsuitable for participation in the study in the opinion of the investigator/physician.

In order to receive Dose 2, subjects must not fulfil any of the above criteria.

9.3.6 Restrictions

The following restriction must be respected by the study subjects during participation in the study:

- Contraception (Women): A woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.

  A WOCBP can participate in the study if practising abstinence (only allowed when this is the preferred and usual lifestyle of the subject)

  OR
  is willing to use a highly effective method of contraception with a *failure rate of < 1%* to prevent pregnancy (from at least 4 weeks prior to dose until completion of the main study, i.e. the follow-up visit 7 days after the last dose):

  - combined (oestrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation (oral, intravaginal, transdermal)
  - progestogen-only hormonal contraception associated with inhibition of ovulation (oral, injectable, implantable), intrauterine device (IUD)
  - intrauterine hormone-releasing system (IUS)

- Contraception (Men):

- With a female partner of childbearing potential or pregnant female partner, men must remain abstinent or use a condom during the treatment period and 7 days after the final dose of IMP. Men must refrain from donating sperm during the same period. Periodic abstinence (e.g. calendar, ovulation, symptothermal or postovulation methods) and withdrawal are not adequate methods for preventing drug exposure.

- Blood donation: The subjects participating in the main study must not donate blood or plasma during the study until 1 month after the last visit. The subjects participating in the exploratory study must not donate blood or plasma during the study until 3 months after the last visit.

- Fasting: The subject should not eat or drink for at least one hour before or after ingestion of the study vaccine

9.3.7 Removal of subjects from therapy or assessment

9.3.7.1 Subject withdrawal criteria and replacement

A study subject should be withdrawn from the study treatment if, in the opinion of the Investigator, it is medically necessary, or if it is the expressed wish of the subject.

Irrespective of the reason for withdrawal and whenever possible, the subject should be examined. Relevant laboratory test samples should be obtained, and all relevant assessments should be completed, preferably according to the scheme for the final assessment. The CRF should be completed as far as possible and checked/verified by the Monitor.

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

- Severe non-compliance to the Study Protocol procedures, as judged by the Investigator and/or SBH
- Subject is lost to follow-up
- Significant AEs posing a risk for the subject, as judged by the Investigator and/or SBH

A withdrawn subject is not allowed to re-enter into the trial.

9.4 Treatments

The wet formulation consists of a liquid suspension of inactivated bacteria (ETEX 21-24) and LCTB*A* in one vial, freeze-dried dmLT adjuvant in a second vial, and effervescent buffer granules in a separate sachet. Prior to administration, the buffer is dissolved in 150 ml tap water, followed by the addition of the content of the vaccine vial (inactivated bacteria mixed with LCTB*A*) and reconstituted and diluted adjuvant dmLT from the second vial.

In the partially dried formulation, dmLT and LCTB*A* are spray-dried and mixed with the buffer granules and stabilizing excipients in a sachet. Prior to administration, the content of the buffer sachet (buffer, dmLT, and LCTB*A*) is dissolved in 150 ml tap water, followed by the addition of a liquid suspension of inactivated bacteria (ETEX 21-24).

The identity of the different components of the two vaccine formulations are described below.

9.4.1 Identity of Investigational Medicinal Product (IMP)

9.4.1.1 Inactivated *E. coli ETEX 21*

The *E. coli* ETEX 21 strain was developed using a recombinant plasmid expressing the entire CFA/I operon [redacted]

9.4.1.2 Inactivated *E. coli ETEX 22*

The *E. coli* ETEX 22 strain was developed using a recombinant plasmid expressing the entire CS3 operon [redacted]

9.4.1.3 Inactivated *E. coli ETEX 23*

The *E. coli* ETEX 23 strain was developed using a recombinant plasmid expressing the entire CS5 operon [redacted]

9.4.1.4 Inactivated *E. coli ETEX 24*

The *E. coli* ETEX 24 strain was developed using a recombinant plasmid expressing the entire CS6 operon [redacted]

9.4.1.5 LCTB*A* protein

LCTB*A* is a hybrid protein between the B-subunit of the *E. coli* heat-labile enterotoxin (LTB) and the B-subunit of the cholera toxin (CTB). [redacted]

9.4.1.6 Oral dmLT adjuvant

LT(R192G/L211A), or dmLT is a derivative of wild-type enterotoxigenic *E. coli* heat-labile enterotoxin that has been genetically modified by replacing the arginine at amino acid position 192 with glycine and the leucine at amino acid position 211 with alanine [25]. [redacted]

The protein dmLT has been manufactured under cGMP at [redacted]. In addition, the stability testing of this clinical lot of dmLT will continue over the course of the clinical phase of this Phase II trial.

9.4.1.7 Sodium bicarbonate protective buffer powder [redacted] used in the Wet formulation

Protective buffer is used to neutralize gastric acidity upon ingestion of vaccine. The dried powder is supplied in moisture-proof sachets (5.6 g/sachet).

For use, the carbonate buffer is dissolved in 150 ml of potable water. The Sodium hydrogen carbonate buffer has been produced by [redacted].

9.4.1.8 dmLT LCTB*A* spray-dried preparation

The dmLT spray-dried preparation was produced under GMP at [redacted].

9.4.1.9 dmLT LCTB*A* spray-dried powder together with effervescent powder and excipients.

The complete dmLT/LCTB*A* effervescent powder was produced under GMP at [redacted]

Protective buffer is used to neutralize gastric acidity upon ingestion of vaccine in particular to protect the dmLT against degradation. The dried powder is supplied in moisture-proof sachets (10.84 g/sachet).

For use, the carbonate buffer is dissolved in 150 ml of potable water.

9.4.1.10 Product(s) to be tested and supply (where applicable)

9.4.1.10.1 Wet formulation

Product name and applicable formulation(s)	***Manufacturer***	***Details of product (approved for use/under development), GMP guarantee, supply and availability – see details above)***	***QP release***
Freeze-dried formulation of dmLT	[redacted]	GMP guarantee, contracted	[redacted]
Wet formulation of CFA/I, CS3, CS5, CS6 and LCTB*A*	[redacted]	GMP guarantee, contracted	[redacted]

9.4.1.10.2 Partially dried formulation

Product name and applicable formulation(s)	***Manufacturer***	***Details of product (approved for use/under development), GMP guarantee, supply and availability – see details above)***	***QP release***
Spray-dried formulation of LCTB*A*, dmLT mixed with buffer	[redacted]	GMP guarantee, contracted	[redacted]
Wet formulation of CFA/I, CS3, CS5, CS6	[redacted]	GMP guarantee, contracted	[redacted]

9.4.1.11 Investigational Product:

New partially dried formulation		**Wet formulation**	
Contents	**Adult dose (mg)**	**Contents**	**Adult dose (mg)**
Part 1: dry powder	[redacted]	**Part 1: dry powder**	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]
[redacted]	[redacted]	[redacted]	[redacted]

	[redacted]			[redacted]	
Part 2: Liquid	[redacted]		**Part 2: Liquid**	[redacted]	
Part 3: liquid	[redacted]		**Part 3: liquid**	[redacted]	

				[redacted]	
Total volume of ETVAX®	[redacted]		**Total volume of ETVAX®**	[redacted]	

NA; not applicable

*; In the wet formulation, dmLT is pipetted into the 150ml of reconstituted buffer containing the vaccine

9.4.2 Packaging, labelling and storage of IMP

9.4.2.1 Packaging

The vaccine vials for both formulations are packed in boxes of 10 vials/box.

The effervescent powder for the wet formulation is packed in boxes of 20 sachets/box.

The dmLT vials for the wet formulation are packed in cardboard boxes with 30-50 vials/box.

The spray-dried formulation of LCTB*A* and dmLT mixed with buffer for the partially dried formulation is packed in boxes of 10-30 sachets/box.

9.4.2.2 Labelling

All IMP will be shipped directly to the study sites or to a local distribution site, according to local requirements.

Labels will comply with applicable Good Manufacturing Practice (GMP) requirements (EudraLex *VOLUME 4, Good manufacturing practices, ANNEX 13, Manufacture of investigational medicinal, products, February 2010, section 26*).

9.4.2.3 Storage

All study medication must be stored in a safe place with limited access, at the temperature given on the labels. Temperature logs should be kept for the area where the IMP is stored at the clinic. The temperature should be noted on a daily basis (working days only unless automatic readings are available).

- Vaccine vials shall be stored at 2-8°C.
- Buffer sachets for the wet formulation shall be stored at 2-25°C.
- Buffer sachets for new partly dry formulation shall be stored at 2-8°C.
- dmLT vials shall be stored at -20±5°C.
- Reconstituted diluted (200 µg/ml) dmLT shall be stored at 2-8°C. Maximum storage time is 48 hours.

9.4.3 Doses and treatment regimens

Both vaccine formulations should be administered only by the oral route. The vaccine will be administered orally twice to each subject at a target interval of 14 days. If the subject is unable to come for the second dose at the third study visit on day 15 due to *e.g.* illness or rules regarding quarantine due to the COVID-19 epidemic, the second vaccine dose can be administered up to 28 days after the first dose. A prolonged interval between vaccine doses did not affect the serum responses to the highly homologous CTB component of the cholera vaccine Dukoral when tested in Swedish volunteers [32]. Thus, serum CTB-specific IgA and IgA responses were comparable in both magnitude and frequency in subjects receiving Dukoral 14 days or 28-48 days apart.

9.4.3.1 Preparation and administration of investigational products

To maintain blinding of the study, the investigational products will be prepared by un-blinded trained nurses on the day of vaccination (Day 1 and day 15) at the clinic.

NOTE: The vaccine will be given to the participants by a blinded study nurse.

Detailed instructions of vaccine preparation for both formulations, including dmLT reconstitution and preparation of stock dmLT will be provided in a separate IMP manual.

9.4.3.1.1 Preparation of Wet formulation

9.4.3.1.1.1 dmLT preparation

A dmLT vial is retrieved from the freezer and thawed at room temperature. 700 µl of water for injection is added to reconstitute the dmLT. After careful swirling of the ampoule 500 to 700 µl are removed from the vial to an Eppendorf tube labelled “Stock dmLT”. A 1/5 dilution of this stock is done in PBS, resulting in a 200 µg/ml solution. From this preparation 50 µl (10 µg) is pipetted into the effervescent buffer vaccine preparation before administration to volunteers.

9.4.3.1.1.2 Preparation of complete Wet formulation

The vaccine supplied as a liquid, is mixed with the 150 ml of sodium bicarbonate buffer solution on the day of preparation for use on dosing day. The time of investigational product preparation will be recorded on the appropriate Accountability Record. The vaccine dose in sodium bicarbonate buffer can be stored at room temperature for up to 90 minutes prior to dosing. The buffer is used to prevent degradation of LCTB*A* hybrid protein, dmLT and sensitive CF antigens by the gastric acid.

Just prior to administration 10 µg of dmLT is added by pipette (50 µl). After addition of dmLT, the vaccine should be used within 30 minutes.

9.4.3.1.2 Preparation of partially dry formulation

The partially dry formulation of vaccine is prepared by adding the effervescent powder containing the dmLT and LCTB*A* to 150 ml of water (the time is recorded), after mixing the content of the vaccine vial is added to the mixture and the vaccine is administered to the volunteer within 30 minutes after adding the buffer powder to the water. The buffer is used to prevent degradation of LCTB*A* hybrid protein, dmLT and sensitive CF antigens by the gastric acid.

9.4.3.2 Vaccine oral solution

Full dose of ETVAX®; a tetravalent ETEC vaccine including LCTB*A* + 10 µg double mutant LT (dmLT) adjuvant and effervescent powder for oral solution

9.4.4 Product accountability

The IMP will be released to the study site(s) after approvals of the study protocol have been received from the IEC and the Competent Authority.

In order to maintain blinding of the study, the IMP preparation procedure will be performed by an un-blinded member of the site staff, not otherwise involved in the conduct of the study. The person who will prepare the IMP needs to be a Registered Nurse (RN). The preparation will be observed by an additional un-blinded person. Both the nurse preparing the vaccine and the observer need to sign the vaccine preparation documentation log.

The study site will maintain a Drug Dispensing Log detailing the dates and quantities of IMP received, prepared, administered/dispensed to each subject as well as study medication returned or destroyed at the end of the study. Any discrepancies between dispensed and returned products must be explained and documented. Products deliberately and/or accidentally destroyed by the Investigator or the subject must be accounted for.

All drug accountability procedures, including cold chain monitoring will be documented and will be responsible of the unblinded study staff.

The *unblinded* Monitor will verify IMP accountability and make sure that all unused IMP is adequately destroyed/returned and documented.

9.4.5 Method of assigning subjects to treatment groups

The randomization list will be generated by the [redacted] biostatistician and the original randomization list will be kept at [redacted]. Subjects will be randomized to treatment with the wet formulation or new partially dry formulation of the vaccine (1:1).

9.4.6 Blinding

This is a double-blind study and the allocation of treatments will not be disclosed until clean file has been declared and the database has been locked for primary and secondary endpoint analyses.

Hence neither the personnel directly involved in taking care of the study subjects or evaluating the results, nor the subjects themselves, will be informed which of the two different formulations of vaccine that is given. The two formulations will have identical appearance and the same smell.

However, the person who prepares the vaccines immediately before immunization and the observer will be unblinded. Theses persons will not be directly involved in evaluation of adverse events or laboratory results.

The unblinded [redacted] Monitor will visit the investigational site to review documents and records related to the preparation of the vaccine, i.e. information unblinded to treatment allocation.

[redacted] pharmacovigilance personnel will be un-blinded for SUSAR reporting purposes but are not involved in data analyses.

9.4.7 Emergency decoding of blinded treatment

The treatment code may only be broken by the study medical staff in case of emergency when knowledge of the treatment received is necessary for the proper medical management of the subject. The code breaking procedure should be carefully documented.

Decoding of the blinded treatment must be reported to SBH.

9.4.8 Prior and concomitant therapy

Therapies considered necessary for the subject's welfare may be given at the discretion of the Investigator.

All concomitant therapy used during and within 3 months prior to the study period must be recorded in the CRF. No other drug under investigation may be used concomitantly with the study medication.

The subjects must not participate concurrently in any other clinical study.

9.4.9 Continuation of treatment

This is a bridging study comparing two formulations of the unregistered product ETVAX® and thus there will be no treatment with ETVAX® available after end of study participation.

9.4.10 Treatment compliance

IMP will be administered at the research clinic (CTC) under medical supervision to ensure compliance.

9.5 Study assessments

The timing and frequency of study visits and assessments are presented in the schedule of events in Table 9.1 and 9.2.

Each study assessment is described in the sections below.

9.5.1 Demographics and other baseline characteristics

9.5.1.1 Informed consent

Signed informed consent must be obtained before any screening procedures are initiated. The informed consent procedure is further described in Section 5.3.

9.5.1.2 Demographic information

The following demographic data will be recorded: race, ethnic origin, gender and date of birth.

9.5.1.3 Medical/surgical history

Relevant medical/surgical history will be obtained by interview in order to verify that the eligibility criteria are met.

In addition, information regarding COVID-19 (infections and vaccinations) as well as detailed travel history to ETEC and cholera endemic countries will be collected, primarily to support evaluation of the exploratory data. This information will not be captured in the CRF, but in a separate document.

9.5.1.4 Prior and concomitant medications

Medication history (prior medications) from 3 months prior to study start until administration of the first dose of IMP will be recorded in the CRF. Prescription medications, Over-the-counter (OTC) medications, and herbal products should be asked for. Any medication used from first administration of IMP until the last follow-up assessment (concomitant medication) will be recorded.

Prior and concomitant medications will be coded according to the WHO Anatomic Therapeutic Chemical (ATC) classification system.

9.5.2 Clinical efficacy assessments

9.5.2.1 Seroconversion rates of IgA and/or IgG anti-LTB antibodies in serum.

IgA and IgG antibodies specific for LTB will be measured in serum samples collected on visit 2 and 4 using an Enzyme Linked Immunosorbent (ELISA) assay. The analyses will be performed by technical laboratory personnel at the [redacted], according to standardized laboratory procedures. The seroconversion rate for IgA and IgG will be determined as the frequencies of subjects having a ≥2 increase in LTB-specific antibody levels in post compared to pre-vaccination samples. Specifically, this will be determined by dividing the antibody titer measured in samples collected 7 days after the second vaccination (Visit 4) with the titer measured in samples collected before vaccination (Visit 2). Samples collected before and after immunization will always be analyzed on the same ELISA-plate. Fold rises in ELISA titers ≥2 will be considered as significant responses for both IgA and IgG.

9.5.3 Safety laboratory assessments

There will be no safety laboratory assessments performed in this study.

9.5.4 Clinical safety assessments

9.5.4.1 Physical examination

A complete physical examination will include assessments of the head, eyes, ears, nose, throat, skin, thyroid, neurological, lungs, cardiovascular, abdomen (liver and spleen), lymph nodes and extremities.

9.5.4.2 Vital signs

Systolic and diastolic blood pressure and heart rate will be measured in the supine position after 10 minutes of rest. Body temperature will be measured in the ear using a digital thermometer.

9.5.4.3 Subject Diary

The study diaries will contain questions regarding adverse events and current medication. The adverse event and medication diaries should be filled in by the subjects on the day of vaccination and five subsequent days. Information about adverse events and medication obtained from the diaries will be verified by interviewing the subject at the following clinic visit and will then be registered in the CRFs by the study nurse/physician, i.e data from study diaries will not be transcribed word by word into the eCRF.
The following solicited AEs will be included in the diary: fever, nausea, vomiting, abdominal pain and loose stools/diarrhea.

9.5.5 Exploratory assessments

All serum samples will be analyzed for LTB specific antibodies using an electrochemiluminescence (ECL) assay based on the Meso Scale Discovery platform in parallel to the ELISA IgA assay [14, 15]. The ECL results will be reported as exploratory data. Serum samples will also be analyzed for antibodies against additional antigens expressed by ETEC bacteria, such as O78 LPS, by ELISA, ECL or similar methods.

Memory cell responses will be evaluated in subsets of subjects using analysis tools recently established for assessment of responses to a number of different infections and vaccines at the [redacted]. These exploratory analyses will be used to validate the new methods for analysis of long-term immunity to ETEC in order to provide additional analysis tools to be used in subsequent ETEC vaccine trials and basic research studies.

Effector T- and B-cell (plasmablast) responses will be analyzed in 40 subjects (20 immunized with the wet and 20 with the partially dry vaccine formulation). Memory T- and B-cell analysis will be performed in 20 subjects (10 immunized with the wet and 10 with the new partially dry vaccine formulation). Memory B cell responses will be assessed on day 30±7 and 75-125 after the second dose by stimulating PBMCs with substances that transform memory B cells into antibody secreting plasmablasts. The production of IgA and IgG antibodies specific for LTB and CFs will be measured in culture supernatants by ECL and/or ELISA. The antigen specificity and isotype distribution of antibodies produced by activated memory B cells will be compared to serum antibody responses at all analysis time points after the second vaccination (day 5±1, 6-10, 30±7 and 75-125) and to antibodies secreted by plasmablasts induced to circulate shortly after the second immunization (day 5±1).

Effector and memory T cell responses will be assessed by flow cytometric analysis of activation markers on T helper cell subsets before and after stimulation of cells with LTB and CFs. The production of cytokines characteristic for different T cell subsets will be evaluated by flow cytometry, ELISA, ECL or other highly sensitive immunoassays. T cell responses will be compared in samples collected before vaccination and after the second dose (day 5±1, 6-10, 30±7 and 75-125). The relation between different types of effector T cell responses and induction of early plasmablast and serum antibody responses (day 5±1 and 6-10) and memory B and T cell responses (day 30±7 and 75-125) will be analyzed.

To check the general immune status of the volunteers and their ability to form long lived memory cells in relation to the ETEC specific immune responses measured after vaccination, immune responses to other common infections and vaccinations will be measured, such as cytomegalovirus, measles, SARS-CoV-2 and tetanus toxoid. Responses will be measured in samples from both the main study and the explorative study with techniques similar to those used to analyze ETEC specific immune responses, such as ELISA, electrochemiluminescence and flow cytometry.

9.5.6 Adverse events

9.5.6.1 Definitions

Adverse Event (AE)

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

A *baseline event* is any medical event in a clinical study subject that occurs after signing the ICF up until the (first) administration of IMP.

Clarifications:

Diagnostic and therapeutic non-invasive and invasive procedures, such as surgery, should not be reported as AEs. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an AE. For example, an acute appendicitis that begins during the AE reporting period should be reported as "acute appendicitis" and the resulting appendectomy noticed under *Comments*. Pre-study conditions, which led to elective surgery during the time of the study, are not to be reported as AEs.

If an abnormal vital sign is associated with corresponding clinical signs and symptoms, the sign/symptom should be reported as the AE and the associated laboratory result or vital sign should be considered additional information that is to be collected in the e-CRF.

Serious Adverse Event (SAE)

An SAE is any AE that:

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

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

Planned hospitalisations or surgical interventions for a condition that existed before the subject signed the ICF and that did not change in intensity are not SAEs.

If there is any doubt as to whether an AE meets the definition of an SAE, a conservative viewpoint must be taken, and the AE must be reported as an SAE.

Serious Adverse Reaction (SAR)

The term SAR is to be used whenever either the Investigator or SBH or designee assessed the SAE as possibly or probably related to the IMP.

Suspected Unexpected Serious Adverse Reaction (SUSAR)

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

9.5.6.2 Assessment of severity/intensity

The Investigator must assess the *severity/intensity* of an AE using the following definitions, and record it on the *Adverse Event Form* in the CRF:

Mild	The AE does not interfere in a significant manner with the subject's normal functioning level. It may be an annoyance.
Moderate	The AE produces some impairment of function but not hazardous to health. It is uncomfortable and/or an embarrassment.
Severe	The AE produces significant impairment of functioning or incapacitation and/or it is a hazard to the subject.

If an AE changes in severity, the most severe intensity should be recorded for that AE; i.e. the AE should not be recorded twice in the eCRF.
For severity grading of AEs, please also see Appendix 3, Clinical Toxicity Grading.

9.5.6.3 Assessment of causal relationship

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

Probably	–	*Probably related* – the AE has a strong temporal relationship to the IMP or recurs on re-challenge, and another aetiology is unlikely or significantly less likely.
Possibly	–	*Possibly related* – the AE has a suggestive temporal relationship to the IMP, and an alternative aetiology is equally or less likely.
Unlikely	–	*Unlikely related* – the AE has no temporal relationship to the IMP or is due to underlying/concurrent illness or effect of another drug (that is, a causal relationship between the IMP and the AE is unlikely).

An AE is considered causally related to the use of the IMP when the causality assessment is *probably* or *possibly*.

For a baseline event, a causality assessment is not relevant.

9.5.6.4 Assessment of Outcome

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

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

9.5.6.5 Collecting and recording of AEs

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

- AEs spontaneously reported by the subject
- AEs observed by the Investigator or medical personnel
- AEs elicited based on non-leading questions from the Investigator or medical personnel
- AEs obtained from Subject Diaries, as described in section 9.5.4.3

Collection of baseline events starts after the subject signs the ICF and continues until the first intervention with the IMP.

AE collection starts with the first intervention with the IMP and continues until the last follow-up assessment. Any AE with start date on the day of first IMP intervention must be recorded with start time.

At the Follow-up Visit, information on new AEs or SAEs, if any, and stop dates for AEs recorded and on-going during the dosing period must be recorded.

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

No distinction should be made between the investigational product and the reference product regarding reporting of SAEs as long as the code is not broken.

Starting from administration of first dose of IMP, all SAEs must be reported by the Investigator to [redacted] Pharmacovigilance, the Monitor and SBH within 24 hours of knowledge of the event, regardless of the time that may have elapsed from the time the event occurred to when the Investigator first learns of it.

All SAEs should be reported to [redacted] Pharmacovigilance:

E-mail: pharmacovigilance@pharmassist.se

The Initial Report should contain as a minimum the following information:

- Subject identification
- Treatment specification (to be expressed in a blinded fashion, e.g. "Treatment A/Treatment B")
- AE diagnosis
- Time specification for the medical event
- Name of the original reporter
- Seriousness classification

A Serious Adverse Event Report Form must also be completed, signed by the Investigator and submitted to [redacted] Pharmacovigilance, the Monitor and Sponsor no later than three calendar days after the initial information was received. Apart from the information above, this Follow-up Report should also contain the following information:

- assessment of severity
- assessment of causality

Only SAEs that are both unexpected and related to the investigational product(s), Suspected Unexpected Serious Adverse Reactions (SUSARs), are subject to expedited reporting. SBH are responsible for informing all Investigators concerned of relevant information about SUSARs that could adversely affect the safety of subjects.
Further details are provided in a separate Safety Management Plan.

9.5.6.6 Exceptions from expedited reporting of SAEs

An event need not to be reported as an SAE if it represents only a relapse or an expected change, or progression of a pre-existing condition and is without any other symptoms or signs than those present before treatment. This event needs only to be reported as an AE and should be described in the Study Protocol.

AEs must be followed up until resolution or the follow-up assessment, whichever comes first. At the Follow-up Visit, information on new AEs, if any, and stop dates for previously reported AEs must be recorded.

It is the responsibility of the Investigator to follow up on all SAEs until the subject has recovered, stabilized, or recovered with sequelae, and to report to SBH all relevant new information using the same procedures and timelines as those for the initial report. Relevant information includes discharge summaries, autopsy reports, and medical consultation.

SAEs spontaneously reported by a subject to the Investigator within 30 days after the last follow-up assessment should be reported to SBH even after the clinical investigation has been finished,

if, in the judgment of the Investigator, there might be an association between the event and the previous use of the IMP or as a result of the investigation procedures.

9.5.6.7 Procedures in case of pregnancy

In case of pregnancy or suspicion of possible pregnancy, the subject must be discontinued from further dosing in the study. Pregnancy itself is not regarded as an AE unless there is a suspicion that the IMP may have interfered with the effectiveness of the contraceptive medication. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, normal birth or congenital abnormality) must be followed up and documented even after the Subject was discontinued from the study.

All events of congenital abnormalities/birth defects are SAEs. Spontaneous miscarriages should also be reported and handled as AEs. Any pregnancy revealed during the study must be reported to SBH and the Monitor on the pregnancy report form. All outcomes of pregnancy must be reported to SBH and the Monitor on the pregnancy outcomes report form.

9.5.6.8 Coding of AEs

All AEs will be coded according to Medical Dictionary for Regulatory Activities (MedDRA) by [redacted].

9.5.7 Appropriateness of measurements

Standard safety assessment methods are used in this study and are very similar to procedures performed to monitor vaccine safety in a previous Phase IIb study of adult Finnish travellers before they left Finland to go to Benin in West Africa (OEV-123, EudraCT No.: 2016-002690-35).

Due to difficulties to get a reliable measurement of immune responses to ETEC CFs in serum of non-primed (i.e. previously not immunized nor infected) Swedish individuals, only the immune response rates to LTB will be measured. For oral vaccines, the responses are evoked in the gut and only some immune responses, e.g. against LTB for ETVAX®, are detectable in serum of immunised individuals [6, 12]. Serum IgA responses to LTB correlate significantly with IgA responses measured in mucosal samples [12]. LTB-specific serum antibody levels measured by ELISA also closely reflect the neutralization capacity of the serum antibodies [12]. ELISA assays to measure LTB-specific IgA and IgG antibodies are well-established and have previously been used to evaluate antibody responses induced by ETVAX® vaccination in Swedish volunteers and in Bangladeshi adults and children [11-15]. The ELISA method used to measure antibodies in this trial has been validated by comparisons with ECL assays in previous studies [14]. Exploratory analyses of serum antibodies will also be performed using the ECL method. A cut-off of 2 for seroconversion has been used in many previous studies of oral vaccines, including all previous trials of ETVAX® .

9.6 Data quality assurance

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

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

9.6.1 Monitoring

The investigational site will be visited both by the *blinded* Monitor and/or the *blinded* [redacted] Project Manager overseeing monitoring activities, as well as the *unblinded* Monitor periodically at times agreed on by the Investigator. It is the function of the Monitor to ascertain that all aspects of the CSP are complied with and that the conduct of the investigation conforms to applicable regulatory requirements and established rules for GCP.

At the time of each monitoring visit, the *blinded* Monitor will review the CRFs to ascertain that all items have been completed and that the data provided are accurate and obtained in the manner specified in the CSP.

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

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

The *unblinded* Monitor will visit the investigational site to review documents and records related to the preparation of the vaccine, i.e. information unblinded to treatment allocation.

A risk-based approach will be developed for the monitoring visits. Further details are provided in a separate Monitoring Plan.

9.6.2 Audits and inspections

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

The Investigator is required to inform SBH immediately of an inspection requested by the Competent Authority (e.g. LV).

9.6.3 Case Report Forms (CRFs)

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

All data must be entered in English. The CRFs should always reflect the latest observations made during the subject's participating in the study. Therefore, the CRFs should be completed as soon as possible during or after the subject's visit.

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

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

Please see section 9.8 for further details.

9.6.4 Source Data

A separate source data document will be generated for the site before start of enrolment, specifying the location of the source of derived information appearing in the CRF. This document must be signed by the Principal Investigator and the Monitor to confirm agreement before start of recruitment.

The Investigator should guarantee access to source documents to the Monitor, Competent Authority (LV) and the IEC (EPM), if required.

9.6.5 Training of study staff

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

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

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

9.7 Statistical methods and determination of sample size

General descriptive statistics for categorial variables will be number of observations (n) and proportion (%) within treatment group. General descriptive statistics for continuous variables will be number of observations (n), Mean, Quartile 1 (Q1), Median, Quartile 3 (Q3), Geometric Mean, Standard deviation (SD) and Range (Min, Max).

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

9.7.1 Description of study population

9.7.1.1 Demographics and baseline characteristics

Descriptive statistics of demographics and other baseline characteristics will be presented for each vaccination group.

9.7.1.2 Medical/surgical history and concomitant medication

Medical/surgical history and prior/concomitant medications will be presented by descriptive statistics for each vaccination group.

9.7.1.3 Analysis of efficacy (Seroconversion rates of IgA and/or IgG anti-LTB antibodies in serum)

The primary endpoint, response, will be defined as "yes" for patients with at least a 2-fold increase in IgA and/or IgG antibody levels against LTB in serum between pre- and post-immunization samples (Visit 4 versus Visit 2).

The response rates (seroconversion rates) within treatment groups, p_{wet} and $p_{partially\_dried}$, will be estimated as the proportion of responders (response = "yes") in each treatment group.
The primary analysis of the study seeks to compare the seroconversion rates using the difference

$d = p_{partially\_dried} - p_{wet}$.

The following non-inferiority hypothesis will be tested, using a -15% non-inferiority limit (on an absolute scale):

H_0: The seroconversion rate of the partially dried group is lower than that of the wet group, $d < -0.15$

H_A: The seroconversion rate of the partially dried group is higher than or equal to that of the wet group, $d \geq -0.15$

A two-sided 95% confidence interval (CI) for d will be calculated by normal approximation. The lower limit of the CI will be compared to the non-inferiority limit -0.15. If the lower CI limit is larger than or equal to the non-inferiority limit, the null hypothesis will be rejected, and the result will be taken as supporting non-inferiority of the partially dried formulation compared to the wet formulation.

The normal approximation requires at least 5 patients in each cell of the Treatment by Vaccine response contingency table. If assumptions are not fulfilled, a non-parametric CI will be used instead.

The primary endpoint will be summarized for the full analysis set (FAS) and the per protocol analysis set (PPAS). The primary endpoint analysis of PPAS will be considered the primary analysis of the study.

The primary endpoint, i.e. the proportion of responders with at least a 2-fold increase in LTB-specific IgA and/or IgG antibody levels, will be summarized with descriptive statistics for the

PPAS and FAS. In addition, the proportion of responders with at least a 4-fold increase in IgA and/or IgG antibody levels will be presented. The levels of serum antibodies at visit 2 and visit 4 and the fold rise (magnitude of response) will also be presented for both IgA and IgG.

9.7.2 Analysis of safety

9.7.2.1 Vital signs

Change from baseline in vital signs (blood pressure, heart rate and body temperature) will be presented by individual time courses for each parameter and subject and summarized by dose group.

9.7.2.2 Adverse Events (AEs)

AEs and SAEs will be recorded from the start of IMP administration until the Follow-up Visit. AEs that occur before first treatment with IMP will be reported separately as baseline event.

All AEs will be described in terms of the treatment/dose at which they occurred. AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) and summarized by Preferred Term (PT) and System Organ Class (SOC). The number of subjects reporting AEs, and the number of AEs reported will be presented by dose group. The events will be tabulated by SOC, PT and by severity and relationship to IMP. SAEs will also be presented in separate tabulations. The number of subjects experiencing an AE in connection to each treatment will be presented.

9.7.3 Analysis of exploratory assessment

The exploratory assessments will be reported separately from the Clinical Study Report (CSR). The exploratory assessments may continue after unblinding of the main study.

Results from exploratory analyses will be summarized using descriptive statistics. The following parameters will be included: for categorical variables, the number and percent in each category; for continuous variables, median (with quartiles), range (minimum, maximum), and geometric means. Magnitudes (fold-rises) of serum antibodies (against LTB measured by ECL and against additional bacterial antigens), memory B cell, antibody secreting cell and T cell responses will be determined at each time point post vaccination compared to pre vaccination. Frequencies of subjects responding with ≥2-fold increased levels of antibodies and markers reflecting T cell activity post vaccination compared to pre vaccination will be determined. Assessment of the change from the baseline measurement to a follow-up measurement will be analyzed using the Wilcoxon signed-ranks test, with adjustment for multiple testing using the Holm´s method, when applicable. Correlations between different types of responses will be analyzed using the Spearman test. P values <0.05 will be considered to be statistically significant.

9.7.4 Analysis data sets

9.7.4.1 Full Analysis Set (FAS)

Unless otherwise stated, statistical evaluations will be based on all subjects who have received randomized treatment with IMP and have available data. Evaluations will be done according to actual treatment received regardless of treatment assigned at randomization. A treatment group will consist of all subjects treated with a specific formulation. The FAS data set will be used for the safety and tolerability assessments as well as for the efficacy endpoints.

9.7.4.2 Per Protocol Analysis Set (PPAS)

The PPAS comprises data from all subjects randomized and treated with evaluable efficacy data, and no major protocol deviation that significantly affects the evaluability of the subject in the study. The PPAS will be used for presentation of the primary endpoint.

9.7.4.3 Determination of sample size

Assuming a seroconversion rate of 85% in both the wet and the new formulation, and aiming to prove non-inferiority with margin -15% (on an absolute scale), 126 subjects are needed in each treatment arm to achieve 90% power. This calculation was done with Farrington-Manning's method, assuming that the study will be evaluated by a two-sided 95% confidence interval for the difference between the two treatment groups' seroconversion rates.

A 10% dropout rate is assumed. The target number of patients to be recruited per study arm is therefore **140.**

9.7.5 Statistical/analytical issues

9.7.5.1 Adjustments for covariates

No adjustment for covariates in statistical analyses are planned, as the study is randomized and double-blinded.

9.7.5.2 Handling of dropouts or missing data

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

9.7.5.3 Multi-centre studies

The study will be conducted at one site.

9.7.5.4 Multiple comparison/multiplicity

Only a single primary hypothesis test will be performed, giving no need for multiple testing adjustment. All other tests will be considered descriptive and should be interpreted with caution. Exploratory statistical tests will be adjusted for multiple testing by Holm's method.

9.7.5.5 Active-control studies intended to show equivalence

Not applicable

9.7.5.6 Examination of subgroups

No examination of subgroups is planned

9.7.5.7 Interim analyses and data monitoring

There will be no interim analysis, internal Safety Review Committee or Data Monitoring Committee for this study.

9.8 Data Management

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

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

9.8.1 The web based e-CRF

Clinical data (including AEs and concomitant medications) will be entered into a 21 CFR Part 11-compliant e-CRF [redacted] provided by [redacted]. The e-CRF includes password protection with 2-Factor Authentication and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents, which are to be defined at the site before inclusion of the first subject.

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

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

9.8.2 Entering of data into the e-CRF

All data must be entered in English.

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

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

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

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

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

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

9.8.3 The query process

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

Data Manager will perform data validation checks on data exported from the e-CRF, as defined in the Data Management Plan (DMP).

Queries will be raised in the e-CRF by Monitor or Data Manager respectively if inconsistencies or suspected errors are found. Queries shall be resolved, and data updated if applicable, in a timely manner by authorised study site personnel. The Monitor or Data Manager cannot enter or update data in the e-CRF.

9.8.4 Source documents

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

Source documents are all documents used by the Investigator or hospital that relate to the subject's medical history, that verifies the existence of the subject, the inclusion and exclusion criteria, and all records covering the subject's participation in the study. Source documents include laboratory results, memoranda, material dispensing records, subject files, etc.

The Investigator is responsible for maintaining source documents. These will be made available for inspection by the Monitor at each monitoring visit. All supportive documentation submitted with the e-CRF, such as laboratory or hospital records, should be clearly identified with the study ID and Subject Number. Any personal information, including name, should be removed or

rendered illegible to preserve individual confidentiality.

9.8.5 Audit trail

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

10 EMERGENCY PROCEDURES

10.1 Emergency contacts

In the case of a medical emergency the Investigator must contact the Chief Medical Officer at SBH (see below).

Name	Function in the study	Phone number and e-mail
[redacted]	Medical Advisor	[redacted] E-mail:[redacted]

10.2 Procedures in case of medical emergency

The Investigator is responsible for ensuring that there are procedures and expertise available to cope with medical emergencies during the study.

10.3 Procedures in case of overdose

An overdose is a dose in excess of the dose specified for each cohort in this Clinical Study Protocol.

Over-dosing is highly unlikely to occur in the study since one vaccine and buffer container will be used per person. The risk for over-dosing is most relevant for the dmLT adjuvant component given to subjects receiving the wet/reference vaccine, where dmLT has to be reconstituted and added manually by a pipette to the other vaccine components. Ten-fold higher oral doses dmLT (100 µg) compared to the dose intended in this trial (10 µg) have previously been found to be safe in healthy adults [26]. Furthermore, four-fold higher doses (4 mg) of the LCTB*A* component have previously been given to Swedish volunteers without causing significant AEs [11]. There are no data on overdosing of the bacterial component of ETVAX® in humans. Over-dosing of

any of the vaccine components is expected to give rise to transient gastro-intestinal symptoms such as vomiting, nausea and loose stools.

There is no known antidote and an overdose in a study subject should be monitored closely and treated symptomatically. Any overdose should be recorded as follows:

An overdose with associated AE is recorded as the AE diagnosis/symptoms on the relevant AE modules in the e-CRF.

An overdose without associated symptoms is only reported in the subject´s medical records.

11 STUDY MANAGEMENT

11.1 Changes in the approved study protocol

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

11.2 Study timetable

The end of the clinical part of the study is defined as the last visit of the last subject participating in the investigation.

The study is expected to start in Quarter 3, 2021.
The main part of the study is expected to be completed by Quarter 3, 2022 and the exploratory part by Quarter 4, 2022.

11.3 Discontinuation of the study

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

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

11.4 Reporting and publication of study results

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

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

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

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

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

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

11.5 Disclosure and confidentiality

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

11.6 Archiving

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

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

11.7 Insurance/indemnity

Subjects will be covered through the Swedish Pharmaceutical Insurance (*Läkemedelsförsäkringen) and the Patient Insurance (Patientförsäkringen)*.

11.8 Study agreements

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

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

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12.1 Signature pages

Principal Investigator

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

Investigator Name	*Signature*	*Date*

Sponsor

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

Medical Advisor (Sponsor signatory)

Name	*Signature*	*Date*

12.2 Declaration of Helsinki

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

12.3 Appendix 3: Clinical Toxicity Grading

Event	Reference Range of Normal Values	Grade I – Mild	Grade II – Moderate	Grade III - Severe
General Severity Grading		An adverse event which is relatively mild and transient in nature, but can be an annoyance, and does not interfere with normal activities.	An adverse event which may be uncomfortable but is not hazardous to health. It may be sufficiently discomforting to interfere with normal activities but does not completely prevent them.	An adverse event which is incapacitating and/or it is a hazard to the subject.
Systemic Reactogenicity		Grade I – Mild	Grade II – Moderate	Grade III - Severe
Diarrhea		3 loose/liquid stools during 24 hour period or loose/liquid stools totaling 200- 400 g	4-5 loose/liquid stools during 24 hour period, or loose/liquid stools totaling 401 - 800g	6 or more loose/liquid stools during 24 hour period, or loose/liquid stools totaling > 800g
Fever (Oral, Ear)		≥100.4°F and ≤ 101.1°F (38.0-38.4°C)	>101.1°F and ≤ 102.0°F (38.5-38.9°C)	> 102.0°F (39.0°C)
Vomiting		1-2 episodes within a 24-hour period	3-4 episodes within a 24-hour period	>5 episodes within a 24-hour period