Study Protocol

Immunologic Responses to a Live Attenuated Oral Cholera Vaccine

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Immunologic Responses to a Live Attenuated Oral Cholera Vaccine

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Clinical Trial Principal Investigator:

Nadine Rouphael, MD

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Statement of Compliance

The trial will be carried out in accordance with Good Clinical Practices (GCP) as required by the following:

- United States Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46; 21 CFR Part 50, 21 CFR Part 54, 21 CFR Part 56, and 21 CFR Part 312);

Compliance with these standards provides public assurance that the rights, safety and wellbeing of study subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki.

All key personnel (all individuals responsible for the design and conduct of this trial) have completed Human Subjects Protection Training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations.

Site Investigator:

Signed: _____ Date: _____

Nadine Rouphael, MD, FIDSA
Hope Clinic of the Emory Vaccine Center
Professor of Medicine
Division of Infectious Diseases
Emory University School of Medicine

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Protocol Summary

Title: Immunologic Responses to a Live Attenuated Oral Cholera Vaccine

Population: 50 subjects, 18 to 49 years old, who are in good health and meet the eligibility criteria

Clinical Sites: The Hope Clinic of the Emory Vaccine Center, Emory University Emory GI Clinic/Ambulatory Surgery Center

Study Duration: 5 years

Subject Duration: Approximately 13 months

Description of Agent: One dose delivered orally of the FDA-approved live attenuated cholera vaccine (Vaxchora)

Objectives:

Primary:

- To evaluate the antibody response to the cholera vaccine in healthy subjects.

Secondary:

- To evaluate B cell responses in healthy subjects receiving the live attenuated cholera vaccine.
- To evaluate the safety and reactogenicity in healthy subjects receiving the live attenuated cholera vaccine.

Exploratory:

- To evaluate mucosal cell responses in a subset of the healthy subjects receiving the live attenuated cholera vaccine.

Endpoints:

Primary:

- Level of antibody titers at day 1 (pre-vaccination) and day 29 in serum.

Secondary:

- Levels of plasmablasts at days 1, 8 and 29

- Levels of activated B cells at days 1, 8 and 29
- Levels of memory B cells at days 1 and 29.
- Correlation between antibody titers and the level of plasmablasts, activated B cells, and memory B cells.
- Production and characterization of monoclonal antibodies against *V. cholerae* at day 8 in a subset of subjects.
- Solicited and unsolicited adverse events in healthy subjects receiving cholera vaccine at day 8 and day 29 respectively.
- Serious adverse events in healthy subjects receiving cholera vaccine for the duration of the study.

Exploratory:

- Levels of plasma cells, activated B cells and T cells in small intestinal biopsy samples at day 29 (n=15) or day 90 (n=5).
- Correlation between systemic and mucosal immune responses.
- Production and characterization of monoclonal antibodies against *V. cholerae* from a protected mucosal site.
- Levels of antibody titers at days 90 and 365.
- Levels of plasmablasts at days 11 and 15 in a subset of subjects.
- Levels of activated B cells at days 11 and 15 in a subset of subjects.
- Levels of T cells at days 1 and 29 in a subset of subjects.

KEY ROLES

Principal Investigator:	Nadine Rouphael, MD, FIDSA Division of Infectious Diseases Emory University The Hope Clinic of the Emory Vaccine Center [redacted]
Co-Principal Investigator:	Steven Keilin, MD Division of Digestive Diseases, Emory University School of Medicine, [redacted]
Co-investigator/ Research Project Principal Investigator:	Jens Wrammert, PhD Department of Microbiology and Immunology, School of Medicine, Emory University, [redacted]

Institution: Emory University

1 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 Background Information

Vibrio cholerae causes an acute diarrheal disease responsible for more than 100,000 deaths and affects an estimated 3 to 5 million people annually [1-3]. Recent epidemics in Haiti and Africa illustrate the continued reach of this pathogen [4]. Across the globe, one billion people lack access to safe drinking water and are vulnerable to cholera. The increasing disease burden, and emergence of more virulent strains, suggest that more aggressive approaches to preventing cholera are needed [5-7]. This includes renewed efforts to understand the mechanism of protective immunity against cholera and to improve the protective efficacy of current cholera vaccines.

V. cholerae infection and the need for improved vaccines. V. cholerae is a non-invasive pathogen, and thus represents an excellent model to study the induction and maintenance of mucosal immunity. V. cholerae colonizes the surface of the small intestine and expresses cholera toxin (CT). The enzymatic activity of CT leads to the profound secretory diarrhea that characterizes cholera [8]. There are currently two WHO prequalified, inactivated whole cell cholera vaccines commercially available, Dukoral and Shanchol. Since 2010, the WHO recommends the use of these vaccines [1], combined with other strategies, in cholera endemic areas, but to date this has been poorly implemented. A major reason for this is that the vaccines have significant immunological and practical limitations. Although the vaccines induce protective immunity, the efficacy is moderate and protection wanes rapidly, with poor protective efficacy in children [9-11]. In contrast, natural infection with V. cholerae induces 90-100% protection against re-infection, for up to ten years [12-14]. **Yet it remains poorly understood why infection confers long-lived protection and current vaccines do not.** A live attenuated cholera vaccine, Vaxchora, was approved in the US by the FDA [15]. In contrast to the inactivated whole cell vaccines, this novel vaccine only requires a single dose, making it more practical for travelers to cholera affected areas. In a human challenge study, a protective efficacy of 90% at day 10 and 80% at day 90 was demonstrated [16]. Interestingly, the 4 out of 33 volunteers that had measureable disease after challenge, displayed markedly less diarrhea, and shed three logs less bacteria in stool, arguing that they were partially protected. This is an important development, as with the ever-expanding travel by the population of developed countries, exposure to this severe human pathogen in one of the 69 cholera-endemic countries in Africa, Asia and the Carribean is likely to increase. Finally, it is currently unknown if this vaccine would provide more long-lived protection than that afforded by the inactivated whole killed vaccines that show little protection already two years after vaccination.

Both live attenuated and cholera vaccines induce early antibody and plasmablast responses that are similar in magnitude to that of natural infection [14, 17-21]. However, the magnitude of the plasmablast response does not necessarily correlate with the induction or maintenance of long-term protection, at least in endemic areas[18, 22], suggesting that there is a need to better

understand these early immune responses and define what critical parameters predict a productive B cell response to cholera. We hypothesize that there are critical aspects of the acute plasmablast response induced by V. cholerae infection, in terms of homing potential to the intestinal mucosa and immunoglobulin isotype, repertoire and antigenic specificity/functional characteristics that dictate the protective efficacy and the duration of humoral immune responses, both systemically and in the intestinal mucosa.

V. cholerae as a model system to understand mucosal humoral immunity in humans. Understanding human mucosal immunity and regulation of homing to mucosal tissues is of key importance, not only for V. cholerae, but for many other mucosal infections, such as influenza and HIV. For example, the discovery of broadly neutralizing monoclonal antibodies (mAb) against HIV [23], underscores the importance of clinical studies aimed at understanding humoral immunity at the mucosal barrier. It is necessary to understand mechanisms that determine mucosal homing and long-term survival of plasmacells in this compartment. Although animal models are essential to understand many mechanistic aspects of immunity, cholera is not recapitulated well in any model tested to date. Thus, the most informative approach is to study this pathogen directly in humans. In this proposal, we demonstrate that the study of cholera in humans offers a strong platform for understanding the induction and longevity of humoral immunity, against a pathogen of great importance for human health. In addition, while an infectious episode provides up to ten years of protection from infection, serum antibody titers rapidly wane to undetectable levels, underscoring the importance of studying mucosal immune responses at the site of action for protective immunity against cholera.

Over the last decade we have worked closely with our collaborators at MGH in Boston and at ICDDR'b in Bangladesh, to characterize acute systemic and mucosal B cell responses in V. cholerae infected patients in a cholera-endemic area in Bangladesh [24-26]. Our colleagues there have studied both blood borne [24, 26] and mucosal [20] (EGD) immune responses to both natural infection and against other, non-live vaccines. The studies proposed herein will expand and complement those studies and allow the direct comparison of infection driven immune responses in an endemic and a naïve setting.

In this study, we attempt to better understand the immune responses to the live-attenuated cholera vaccine (Vaxchora) in healthy naïve subjects. Single cell expression cloning will generate a panel of monoclonal antibodies from these patients to further dissect the antibody responses induced by this vaccine. In addition to providing insight into the biology of vaccine induced immune responses, this analysis may also generate reagents with potential diagnostic or even therapeutic potential. Finally, we attempt to better understand the mucosal immune response to the live-attenuated cholera vaccine (Vaxchora) in a subset of subjects.

1.2 Scientific Rationale

The immune response to cholera varies between natural infection and vaccination in terms of magnitude, quality and longevity. Here we attempt to better understand the immune response to cholera vaccination in healthy subjects who are naïve to cholera vaccination and/or infection. As protection against *V. cholerae* is thought to occur at the mucosal surface in the small intestine, we propose to analyze local, mucosal immunity induced by vaccination with the live attenuated cholera vaccine, Vaxchora. This analysis will provide key insight into the dynamics of protective immunity against cholera, and also significantly enhance our understanding of mucosal immunity in general, which is of importance for many other mucosal infections, such as rotavirus, shigella, and HIV infections.

1.3 Potential Risks and Benefits

The study utilizes an FDA-approved live cholera vaccine, Vaxchora. See package insert for full risks and benefits of the vaccine [27].

1.3.1 Potential Risks

The potential risks to all subjects are those associated with oral administration of the live cholera vaccine, possible reactions to the vaccine, and with having blood drawn.

The potential risks of receiving the live cholera vaccine include but are not limited to: tiredness (30%), headache (28%), abdominal pain (18%), nausea/vomiting (17%), lack of appetite (16%), diarrhea (4%), and fever (0.6%) [28]. If any participant develops severe watery diarrhea we will clinically assess the patient and facilitate access to treatment

Clinical trials found no related serious adverse reactions to the Vaxchora vaccine. Rarely, a subject may experience an allergic reaction, which produces rash, hives, or difficulty breathing. Vaccine products have been shown to be shed in stool for at least one week following administration, posing a potential risk. Subjects will be instructed to be diligent with hand washing and avoid contact with infants and immunosuppressed patients as per the vaccine package insert.

Blood sample collection involves transient discomfort and may cause fainting, which is managed by having the subject lie down prior. The blood draw site may bruise, and this can be ameliorated by holding pressure to this site following the blood draw. The sites of blood draw are potential sites of infection, but this risk is made very unlikely by the use of sterile technique.

For the subset of patients (n=30) for which an upper endoscopy biopsy (EGD) is performed, additional risks involve those associated with the EGD procedure. Risks of the EGD procedure include complications related to: sedation, endoscopy, and the biopsy extraction. In a large survey from 1974 that was based on 200,000 upper endoscopies, the overall complication rate was 0.13%, and the mortality rate was 0.004%. Subsequent studies have estimated

complication rates of 0.15% overall and 0.0002% for diagnostic endoscopy without therapeutic maneuvers [29-32]. The risk for the healthy cohort proposed herein are likely less, as the numbers above include patients with advanced age, cardiopulmonary disease, anemia or obesity. Since our cohort is a healthy patient population, we expect our complication rates to be lower.

The most frequent and serious complications of procedural sedation are cardiopulmonary, with risk factors being: advanced age, underlying comorbid illness (especially pulmonary disease), sleep apnea, obesity, anemia, and dementia. The frequency of cardiopulmonary events is associated with increasing American Society of Anesthesiologists scores. Some adverse events due to oversedation include: aspiration, hypoventilation, hypotension, hypoxemia, airway obstruction, vasovagal episodes, and arrhythmias. The overall incidence of cardiopulmonary complications is low. In a prospective survey of 14,149 upper endoscopies and a retrospective study of 21,011 endoscopic procedures, the rates of early cardiopulmonary events were 2 to 5.4 per 1000 cases and mortality rates were 0.3 to 0.5 per 1000 cases, including cases of pneumonia, pulmonary embolism, aspiration, and myocardial infarct [33, 34].

Bleeding rarely occurs following diagnostic upper endoscopy. While the risk may be increased in patients with thrombocytopenia or coagulopathies, diagnostic upper endoscopy is generally thought to be safe in patients with platelet counts as low as 20,000 [32]. The risk is increased with use of anticoagulants and antiplatelet medications.

Upper endoscopy is associated with perforation of the esophagus, stomach and small bowel. It is more common when therapeutic maneuvers are carried out and in patients with esophageal diverticula. The estimated risk of esophageal perforation varies with the procedure being performed [35].

- Diagnostic endoscopy with a flexible endoscope: 0.03%
- Diagnostic endoscopy with a rigid endoscope: 0.11%
- Stricture dilation: 0.09 to 2.2%
- Sclerotherapy: 1 to 5%
- Pneumatic dilation for achalasia: 2 to 6%

The risk of infection related to gastrointestinal endoscopy is low, though there have been cases of hepatitis B, hepatitis C, and bacterial transmission related to breaches in protocols for proper endoscope disinfection, including an outbreak of carbapenem-resistant Enterobacteriaceae associated with improper processing of side-viewing duodenoscopes [36].

1.3.2 Known Potential Benefits

While individuals are generally not at risk for acquiring cholera in the United States, the vaccine can offer protection to subjects if traveling to endemic countries, where individuals who receive this vaccine are likely to receive some level of protection from future *V. cholerae* exposure. The benefit for the study is predominantly scientific, allowing a better understanding of the immune response, in both the blood and in the mucosal tissues, to this particular vaccine.

2 OBJECTIVES

2.1 Study Objectives

Primary:

- To evaluate the antibody response to the cholera vaccine in healthy subjects.

Secondary:

- To evaluate B cell responses in healthy subjects receiving cholera vaccine.
- To evaluate the safety and reactogenicity in healthy subjects receiving cholera vaccine.

Exploratory:

- To evaluate mucosal cell responses in a subset of the healthy subjects receiving cholera vaccine.

2.2 Study Outcome Measures

Primary:

- Level of antibody titers at day 1 (pre-vaccination) and day 29 in serum.

Secondary:

- Levels of plasmablasts at days 1, 8 and 29
- Levels of activated B cells at days 1, 8 and 29
- Levels of memory B cells at days 1 and 29 .
- Correlation between antibody titers and the level of plasmablasts, activated B cells, and memory B cells.
- Production and characterization of monoclonal antibodies at day 8 against *V. cholerae* in a subset of subjects.
- Solicited and unsolicited adverse events in healthy subjects receiving cholera vaccine at day 8 and day 29 respectively.

- Serious adverse events in healthy subjects receiving cholera vaccine for the duration of the study

Exploratory:

- Levels of plasmacells, activated B cells and T cells in small intestinal biopsy samples at day 29 (n=15) or day 90 (n=5).
- Correlation between systemic and mucosal immune responses.
- Production and characterization of monoclonal antibodies against *V. cholerae* from a protected mucosal site.
- Levels of antibody titers at days 90 and 365.
- Levels of plasmablasts at days 11 and 15 in a subset of subjects.
- Levels of activated B cells at days 11 and 15 in a subset of subjects.
- Levels of T cells at days 1 and 29 in a subset of subjects.

3 STUDY DESIGN

This study will be a prospective study, with 50 subjects total, who are between ages 18 and 49 years of age, in good health and meet all eligibility criteria. Each subject will make a total of 7 visits to the Hope Clinic. Subjects will be recruited from the Atlanta metro area. (See appendix). 30 of the subjects will also have two different visits at the GI suite during which small intestinal biopsies will be obtained: one at screening (-45 to -7 days) and the other post vaccination (Group 1 at Day 29 -7 days/ +14 days and Group 2 at day 90 +14 days) by EGD, and will have an additional follow-up phone call 7 days after the procedure. Subjects will be recruited from the Atlanta metro area.

4 STUDY POPULATION

4.1 Selection of the Study Population

The target sample size will be 50 subjects who will receive the Vaxchora live cholera vaccine, of whom 30 will undergo two procedures for small intestinal biopsies (6-12 biopsies per EGD procedure in total): one at screening and the other post vaccination (Group 1 at Day 29 and Group 2 at day 90) by an EGD. With the exception of the inclusion/exclusion criteria, there will be no intentional recruitment of particular ethnic or racial groups. Subjects will be screened for eligibility according to the inclusion/exclusion criteria by history. Informed consent will be obtained for study participation.

4.2 Inclusion/Exclusion Criteria

Subjects eligible to participate shall meet all of the following **inclusion criteria:**

1. Capable of informed consent and provision of written informed consent before any study procedures.

2. Capable of attending all study visits according to the study schedule.

3. Males or females between the ages of 18 and 49 years of age (inclusive).

4. Are in good health, as determined by medical history and targeted physical exam related to this history.

5. Female subjects of childbearing* age must have a negative urine pregnancy test before study vaccination and must use two forms of contraception** to avoid pregnancy within one month of Vaxchora administration.

** Not sterilized via tubal ligation, bilateral oophorectomy, hysterectomy, or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with documented radiological confirmation test at least 90 days after the procedure, and still menstruating or <1 year of the last menses if menopausal..*
***Includes, but is not limited to, non-male sexual relationships, abstinence from sexual intercourse with a male partner, monogamous relationship with vasectomized partner who has been vasectomized for 180 days or more prior to the subject receiving the first study vaccination, barrier methods such as condoms or diaphragms with spermicide or foam, effective intrauterine devices, NuvaRing®, and licensed hormonal methods such as implants, injectables, or oral contraceptives ("the pill).*

Subjects eligible to participate shall not meet any of the following **exclusion criteria**:

1. Have an acute illness within 72 hours before vaccination.

2. Have any acute or chronic medical condition that, in the opinion of the principal investigator, would make vaccination unsafe or interfere with the evaluation of immune response to study vaccination.
3. Have a suppressed immune system as a result of illness, immunosuppressive medication, chemotherapy, or radiation therapy within 3 years prior to study vaccination.
4. Have taken oral or parenteral corticosteroids of any dose within 30 days before study vaccination.
5. Reside with individuals under the age of 2 or with an immunocompromised individuals.
6. Have a known history of autoimmune disease.
7. Have a history of Guillain-Barre Syndrome.
8. Have plans to receive any vaccine from 28 days prior to study vaccination until Day 29.
9. Has previously received a cholera vaccine or have a known history of *V. Cholerae*.
10. Have donated blood or blood products within 56 days before study vaccination, plan to donate blood at any time during the 56-day duration of subject study participation, or plan to donate blood within 56 days after the last blood draw.
11. Have known hypersensitivity or allergy to any component of the vaccine or history of anaphylaxis with a vaccine or vaccine component.
12. Have allergy to tetracycline and/or ciprofloxacin.
13. Are pregnant or breastfeeding or plan to within one month of vaccination.
14. Travelled to a cholera endemic area and had traveler's diarrhea in the previous 5 years.
15. Have abnormal stool pattern (fewer than 3 stools/ week or greater than 2 stools/ day) or regular use of laxatives in the last 6 months.
16. Have current or recent antibiotic use in the past 14 days.
17. Are healthcare workers who have direct contact with patients who are immunocompromised, have unstable medical conditions, or are under the age of 2.
18. Are childcare caregivers who have direct contact with children who are 2 years or younger.
19. Are employed in the food industry.
20. Have received any vaccine within the previous 21 days.
21. History of bleeding disorders or current use of warfarin, aspirin, heparin, nonsteroidal anti-inflammatory drugs (NSAIDs) or other blood thinner/ anticoagulant medications in the past week for subjects undergoing intestinal biopsies.
22. Use of benzodiazepines or narcotics for subjects undergoing intestinal biopsies 4 weeks prior to the procedure

23. Any contraindications to endoscopy/concerns of the anesthesiologist for subjects who agree for EGD/biopsies.
24. BMI > 35 kg/m^2
25. Have a diagnosis of any small bowel disease. This includes but is not limited to inflammatory bowel disease (e.g., Crohn's disease, ulcerative colitis, indeterminate colitis, or microscopic colitis), small bowel obstruction, celiac disease, h/o small bowel resection, small bowel lymphoma, Whipple's disease, primary Intestinal lymphangiectasis, abdominal radiation.
26. Current medications for the treatment of Gastroesophageal reflux disease (GERD) or dyspepsia
27. History of Helicobacter pylori (H. pylori) infection

5.0 **Recruitment**

Recruitment for the study will be done using various recruitment platforms and methods described below:

<u>Posting and distributing IRB approved flyers around the community:</u>

- IRB approved flyers will be posted in and around the community. The flyers will be distributed using methods including but not limited to:
  - Approved flyers will be posted on billboards.
  - Posting and handing flyers to the public in locations such as Malls, Airports, parks, recreation centers, restaurants etc.
- Posting IRB approved Ads on Shuttles and buses – Emory University buses/shuttles, Georgia Tech shuttles/buses, MARTA buses and bus stops
- The Hope Clinic team will take part in community events throughout Atlanta where study staff will talk to attendees about research studies being done at the Hope Clinic, including this study.

The study team will ensure that in locations where required, permission from relevant authorities will be sought before distributing or posting the flyers.

<u>Social media and paid advertising:</u>

- Posting ads on different social media platforms such as Facebook, Instagram, Twitter, Snapchat, TikTok etc. and dating apps containing a link to the Hope Clinic Volunteer Form.
- The Emory Hope Clinic website (A short description of the study will be put on the Hope Clinic website using the same language as the ads).
- IRB approved Ad banners may also be placed on streaming platforms including but not limited to Netflix, Hulu, Disney+ etc.
- We may also partner with cable companies such as AT&T, Xfinity etc. to post paid ads. The ads may be posted in the form of texts, email or banner on cable TV.

Recruiting via email:

- Email blasts of IRB approved email language will be sent out on various listservs e.g., Emory Vaccine Dinner Club, Emory Vaccine Center, CDC etc.
- Email blasts to student listservs including Emory University, Georgia State University, Georgia Tech and UGA. The study team will seek approval from the respective universities registrars and verify the method in which the email blast will be sent out (listservs or individual email).

Registries

- We may also use registries as a recruitment platform for this protocol. Various volunteer registries may be utilized. After potential candidates have been identified from the registry, they may be contacted by their indicated method of contact (email, mailing address or phone/texting) and invited to be screened under this protocol.

Contacting past and present study subjects

- Current and past Hope Clinic subjects (i.e. who have agreed to be contacted in past informed consent forms) may be contacted by calling and emailing. Potential new subjects who will self-refer from recruitment advertisements will be included. All recruitment materials will be IRB approved prior to use. IRB approved advertisements may be placed in local media platforms.

Verbal/written consent is first obtained and then subjects will be interviewed to assess ifthey meet the inclusion and exclusion criteria. They will be enrolled and undergo the screening procedures indicated unless it is discovered that they are not eligible to continue.

5 STUDY PROCEDURES/EVALUATIONS

5.1 Study Procedures

Medical history will be obtained by interview of study subjects on Day 1 (Visit 1) prior to the study vaccination. Subjects will be asked about a known history of significant medical disorders, cancer, immunodeficiency, allergies, psychiatric illness, substance use, and autoimmune diseases.

Medications history will include a review of all current medications and any medications taken in the last 30 days before study vaccination. Medications included in this history will include prescription medication, over-the-counter medication, vitamins, supplements, and prohibited treatments listed in the above Inclusion/Exclusion Criteria section, such as antibiotics within the last 14 days and vaccines.

On Day 1 (Visit 1) and before the study vaccination, a targeted physical examination may be performed by the investigator, who is licensed to make medical diagnoses. At visits 2-7, a targeted physical examination may be performed based on interim health history.

Vital signs (oral temperature, pulse rate, and blood pressure) will be measured at all study visits.

Height and weight will be measured on Day 1 (Visit 1) before study vaccination

Subjects will be observed in the clinic for at least 20 minutes after study vaccination on Day 1 (Visit 1).

5.1.1 Laboratory Evaluations/Assays

Urine pregnancy tests will be performed by the site laboratory on the same day as and prior to study vaccination (Day 1). Results must be negative and known prior to study vaccination.

5.1.2 Special Assays or Procedures

The immunologic assays will be performed at Dr. Wrammert’s Laboratory on the Emory University main campus.

5.1.3 Specimen Collection, Preparation, Handling and Analyses

Blood will be collected at each of the seven study visits as per the Study Schedule in Appendix A.

6 STUDY SCHEDULE

6.1 Screening and Enrollment/Baseline Visit

6.1.1 Screening Visit Day 00 (As needed) (Day -45 to -7 days)

Study subjects will be given a description of the study and have an opportunity to have their questions and concerns addressed by the study principal investigator or designee.

Subjects will be given the informed consent form to read and discuss with study staff, and if they wish to enroll will sign the document before any study vaccination, procedures, or lab draws are performed.

A medical history and targeted physical exam, if necessary, as outlined in the Study Procedures section will be conducted in order to assess for compliance with study inclusion and exclusion criteria.

A list of any medications taken by subjects in the past 30 days and those to be taken for 28 days after vaccinations will be documented on the appropriate study form.

A urine pregnancy test will be conducted for female subjects, and a negative result will be required and be documented on the appropriate study form prior to any study interventions.

Vital signs, including pulse rate, blood pressure, oral temperature, and height and weight will be measured and documented on the appropriate study form.

Group 1 and Group 2 subjects: a procedure for small intestinal biopsies using esophagogastroduodenoscopy will be performed by a gastrointestinal specialist at Emory GI suite. Visit will take 4 hours.

6.1.2 Visit 0a (screening+7) for groups 1 and 2

Groups 1 and 2: a follow-up phone call is made 7 days post procedure to assess for any adverse events from the EGD procedure.

6.1.3 Enrollment Day 01

Study subjects will be given a description of the study and have an opportunity to have their questions and concerns addressed by the study principal investigator or designee.

Subjects will be given the informed consent form to read and discuss with study staff (if no screening visit has been done), and if they wish to enroll will sign the document before any study vaccination, procedures, or lab draws are performed.

A medical history and targeted physical exam, if necessary, as outlined in the Study Procedures section will be conducted in order to assess for compliance with study inclusion and exclusion criteria.

A list of any medications taken by subjects in the past 30 days and those to be taken for 28 days after vaccinations will be documented on the appropriate study form.

A urine pregnancy test will be conducted for female subjects, and a negative result will be required and be documented on the appropriate study form prior to any study interventions.

Vital signs, including pulse rate, blood pressure, oral temperature, and height and weight will be measured and documented on the appropriate study form.

Blood will be collected for baseline study labs as outlined in the Study Procedures section.

A single dose of the FDA-approved oral live cholera vaccine (Vaxchora) will be administered. Eating and drinking should be avoided for one hour before and after vaccine administration. Subjects will be asked to remain at the clinic for 20 minutes to monitor for any reactions or adverse events. Any reactions or adverse events will be documented on the appropriate study form. Subject will be instructed to use adequate hand hygiene to avoid fecal-oral transmission of the vaccine to others.

Subjects will be informed to contact the study clinic should they experience any concerning reactions to the vaccine, and they will be asked to visit the clinic for evaluation should the principal investigator or designee deem the reaction in need of evaluation.

6.2 Follow-up and Final Visits

6.2.1 Visit 02 Day 08 Visit 03 Day 11, Visit 04 Day 15

Interim medical history and targeted physical exam, if necessary, will be conducted.

Medications taken during the interim from the previous visit and any adverse or serious adverse events will be documented on the appropriate study form.

Vital signs, including pulse rate, blood pressure, and oral temperature will be measured and documented on the appropriate study form.

Blood will be collected for immunogenicity analysis.

A review of the memory aid will be done at Visit 02.

Certain follow up visits (D11 and D15) will not be required by PI discretion.

6.2.2 Visit 05 Day 29

Interim medical history and targeted physical exam, if necessary, will be conducted.

Medications taken during the interim from the previous visit and any adverse or serious adverse events will be documented on the appropriate study form.

Vital signs, including pulse rate, blood pressure, and oral temperature will be measured and documented on the appropriate study form.

Blood will be collected for immunogenicity analysis.

Group 1 (n=15): a small intestinal biopsy using esophagogastroduodenoscopy will be performed by a gastrointestinal specialist at Emory GI suite. Visit will take 4 hours. The window for this visit is – 7 days/ + 14 days

6.2.3 Visit 06 Day 90

Interim medical history and targeted physical exam, if necessary, will be conducted.

Any serious adverse event will be documented on the appropriate study form.

Vital signs, including pulse rate, blood pressure, and oral temperature will be measured and documented on the appropriate study form.

Blood will be collected for immunogenicity analysis

Group 2 (n=5): a small intestinal biopsy using esophagogastroduodenoscopy will be performed by a gastrointestinal specialist at Emory GI suite. Visit will take 4 hours. The window for this visit is + 14 days. Visit 05a (EGD +7 days) and Visit 06a (EGD day+7 days)

Group 1 and 2: a follow-up phone call is to be made 7 days post-procedure to assess for any adverse events from the procedure. Visit 07 Day 365

Interim medical history and targeted physical exam, if necessary, will be conducted.

Any serious adverse event will be documented on the appropriate study form.

Vital signs, including pulse rate, blood pressure, and oral temperature will be measured and documented on the appropriate study form.

Blood will be collected for immunogenicity analysis

6.3 Early Termination Visit (if needed)

The following will be performed at any early termination visit, if necessary, for subjects who withdraw or who are withdrawn from the study:

Interim medical history and targeted physical exam, if necessary, will be conducted.

A review of the memory aid will be conducted (if visit occurs within 7 days of vaccination).

Medications taken during the interim from the previous visit will be documented on the appropriate study form (if prior to D29).

Vital signs, including pulse rate, blood pressure, and oral temperature will be measured and documented on the appropriate study form.

Any adverse (prior to D29) or serious adverse events will be documented on the appropriate study form.

Blood will be collected for immunogenicity analysis.

7 STATISTICAL CONSIDERATIONS

The goal of this study is to study the immune responses to the live attenuated cholera vaccine Vaxchora in healthy, cholera infection/vaccination naïve subjects by analysis of the blood and mucosal tissue samples from the small intestine.

Our aim is to include a total of 50 participants who will receive the live attenuated oral cholera vaccine (Vaxchora), of which 30 participants will undergo 2 procedures for small intestinal biopsy via EGD by a gastrointestinal specialist at Emory University Hospital.

The biostatistician will perform descriptive statistics to describe the immune responses to cholera vaccine. As a faculty in the Department of Pediatrics, Dr. Wrammert has access to an excellent Statistical Core that is provided free of charge.

8 QUALITY CONTROL AND QUALITY ASSURANCE

The study will undergo internal quality control and quality assurance per the Hope Clinic standard operating procedures.

9 ETHICS/PROTECTION OF HUMAN SUBJECTS

9.1 Ethical Standard

The site principal investigator will ensure that this trial is conducted in full conformity with the principles of the Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR 46, 21 CFR 50 and 56; 62 Federal Regulations 25691 (1997), if applicable.

9.2 Institutional Review Board

Prior to enrollment of subjects into this trial, the approved protocol and informed consent form will be reviewed and approved by the Emory IRB. The responsible official for the IRB will sign the IRB letter of approval of the protocol prior to the start of this study.

9.3 Informed Consent Process

The site principal investigator and designee will choose subjects in accordance with the eligibility criteria detailed in Section 4. Before any study procedures are performed, subjects must sign an informed consent form that complies with the requirements of 21 CFR Part 50 and 45 CFR 46 and the local IRB.

Informed consent is a process that is initiated prior to an individual agreeing to participate in a study and continuing throughout the individual's study participation. Before any study procedures are performed, subjects will receive a comprehensive explanation of the proposed study procedures and study interventions/products, including the nature and risks of the trial, alternate therapies, any known adverse effects, and the other elements that are part of obtaining proper informed consent. Subjects will also receive a detailed explanation of the proposed use and disclosure of their protected health information, including specifically their serum samples. Subjects will be allowed sufficient time to consider participation in the study, after having the nature and risks of the trial explained to them, and have the opportunity to discuss the trial with their family, friends or legally authorized representative or think about it prior to agreeing to participate.

Informed consent forms describing in detail the study interventions/products, study procedures, risks and possible benefits are given to subjects. The informed consent form must not include any exculpatory statements. Informed consent forms will be IRB-approved and subjects will be asked to read and review the appropriate document. Upon reviewing the appropriate document, the site principal investigator (or designee) will explain the research study to subjects and answer any questions that may arise. Subjects must sign the informed consent form, and written documentation of the informed consent process is required prior to starting any study procedures/interventions being done specifically for the trial, including administering study product.

Study personnel may employ IRB-approved recruitment efforts prior to obtaining the subjects consent; however, before any study procedures are performed to determine protocol eligibility an informed consent form must be signed. Subjects will be given a copy of all informed consent forms that they sign.

This submission seeks approval for two new study flyers intended for print and social media use. Specifically, the flyers will be disseminated, once IRB-approved, in the community, on Emory University Campuses and electronically via listservs and on electronic notification boards. The flyers may also be posted on social media platforms such as Facebook, Instagram, Twitter and craigslist via an official Emory University Facebook account and Instagram Emory Get Involved account.

By signing the informed consent form, subjects agree to complete all evaluations required by the trial, unless the subject withdraws voluntarily, or is withdrawn or terminated from the trial for any reason.

The rights and welfare of subjects will be protected by emphasizing to subjects that the quality of their medical care will not be adversely affected if they decline to participate in or withdraw from this trial.

9.4 Exclusion of Women, Minorities, and Children (Special Populations)

This trial will be inclusive of all adults who meet the Subject Inclusion/Exclusion Criteria, regardless of religion, sex, or ethnic background.

9.5 Subject Confidentiality

Subjects will have code numbers and will not be identified by name. Subject confidentiality is strictly held in trust by principal investigator and the Hope Clinic personnel directly involved in the study. This confidentiality is extended to cover testing of biological samples, in addition to the clinical information relating to participating subjects. The study protocol, documentation, data, and all other information generated will be held in strict confidence.

9.6 Future Use of Stored Specimens

Subjects will be asked for permission to keep any remaining samples for possible use in future research studies, such as examining additional immunological assessments or testing for antibodies against other viruses or bacteria. The samples will not be sold or used directly for production of any commercial product. Human genetic tests will be performed on samples. Each sample will be labeled with a unique tracking number to protect subject’s confidentiality. There are no benefits to subjects in the collection, storage and subsequent research use of specimens. Reports about future research done with subject’s samples will NOT be kept in their health records.

Subjects may be given the option to decide if they want their samples to be used for future research or have their samples destroyed at the end of the study. The subject's decision can be changed at any time prior to the end of the study by notifying the study doctors or nurses in writing. However, if the subject originally consents to future use and subsequently changes his/her decision, any data from a previously collected sample may still be used for this research.

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SUPPLEMENTS/APPENDICES

Study Visit Number	**Screen (D00)**	**(V0a)**	**V01**	**V02**	**V03**	**V04**	**V05**	**(V05a)**	**V06**	**(V06a)**	**V07**
Study Day +/- Window (days)	**-45/-7**	**D00+7**	**D1**	**D8+1**	**D11 optional**	**D15 optional**	**D29 -7/+14**	**EGD+7**	**D90 -4/+7**	**EGD+ 7**	**D365 +/-14**
Obtain Informed Consent[#]	X*		X*+								
Review Eligibility Criteria	X*		X*								
Medical History[+]	X*	X	X*	X	X	X	X	X	X	X	X
Concomitant Medications	X*%	X	X*%	X	X	X	X				
Vital Signs (Oral Temperature, Pulse Rate, and BP)	X*&$		X*&$	X$			X$		X$		
Height and Weight	X*		X*								
Targeted Physical Examination	X*		X*								
Urine Pregnancy Test	X*@		X*@				X*@				
Study Vaccination			X								
20-minute Evaluation Period After Study Vaccination			X								
Venous Blood Collection for Study Assays	X		X	X	X	X	X		X		X
Small intestinal biopsy via EGD	X!						X=		X=		

Memory Aid review				X							
Post-procedural phone call^		X						X		X	
Adverse events	X	X	X	X	X	X	X				
Serious adverse events	X	X	X	X	X	X	X	X	X	X	X

#Prior to study procedures

*Prior to study vaccination.

+Not required if done at the screening visit

%All current medications and medications taken within 30 days prior to day 1 of the study.

&Vital signs assessed on Day 1 (Visit 1) before the study vaccination will be considered as baseline.

$Subjects must not eat or drink anything hot or cold, or smoke within 10 minutes prior to taking oral temperature.

() Targeted physical examination if indicated based on review of complete or interim medical history.

@Must be performed on all female subjects of childbearing potential within 24 hours prior to each study vaccination and results must be negative and known prior to each study vaccination.

+Complete medical history by interview of subjects to be obtained on Day 0 (Visit 01) prior to the first study vaccination and interim medical history by interview of subjects to be obtained at follow-up visits after study vaccination.

!Only for participants agreeing to EGD i.e. Group 1 and 2

= Group 1 (n=25) will undergo small intestinal biopsy on D29 and Group 2 (n=5) subjects will undergo small intestinal biopsy at D90.

^Follow-up phone call post small intestinal biopsies via EGD.

Appendix B: Adverse Events Grading

Vital Signs*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever** (°C) (°F)	 38.0- 38.4 100.4- 101.1	 38.5- 38.9 101.2- 102.0	 39.0- 40.0 102.1- 104.0	 >40.0 >104.0
Tachycardia (beats per minute)	101-115	116-130	>130	ER visit or hospitalization for arrhythmia
Bradycardia*** (beats per minute)	50-54	45-49	<45	ER visit or hospitalization for arrhythmia
Hypertension-systolic (mm Hg)	141- 150	151- 155	>155	ER visit or hospitalization for malignant hypertension
Hypertension-diastolic (mm Hg)	91- 95	96- 100	>100	ER visit or hospitalization for malignant hypertension
Hypotension-systolic (mm Hg)	85-89	80-84	<80	ER visit or hospitalization for hypotensive shock

* Should be at rest for all vital sign measurements

** Oral temperature; no recent hot or cold beverages or smoking

*** When resting heart rate is between 60-100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Systemic (General)	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Nausea/vomiting	No interference with activity or 1-2 episodes/24 hours	Some interference with activity or >2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2-3 loose stools or <400 gms/24 hours	4-5 loose stools or 400-800 gms/24 hours	6 or more watery stools or >800 gms/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever> 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Abdominal pain	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization

Lack of appetite	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization