Protocol Title: Collection of Blood for In Vitro Studies from Healthy Adults Who Have Received Oral Cholera Vaccine (CVD 103-HgR)

Protocol Number: Blood Donor CVD 9000

Sponsored by:

Center for Vaccine Development

University of Maryland School of Medicine

Baltimore, MD

Principal Investigator:

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Version Number: 1.0

October 26, 2018

Statement of Compliance

This study will be carried out in accordance with the United States (US) Code of Federal Regulations (CFR), local regulations, and Good Clinical Practice (GCP) as required by the following

US CFR applicable to clinical studies (45 CFR 46; and 21 CFR including part 50 and 56 concerning informed consent and institutional review board (IRB) regulations and 21 CFR 11 concerning electronic records,)

- International Conference on Harmonisation (ICH) E6 (R1); 62 Federal Register 25691 (1997)
- NIAID Clinical Terms of Award

All individuals responsible for the design and conduct of this study have completed Human Subjects Protection Training and are qualified to be conducting this research prior to the enrollment of any volunteers. Curricula vitae for all investigators and sub-investigators participating in this study are on file in a central facility (21 CFR 312.23 [a] [6] [iii] [b] edition).

Signature Page

The signature below constitutes approval of this protocol and the attachments and provides the required assurances that this study will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements, applicable US federal regulations, and (ICH E6 [R1]) guidelines.

Clinical Principal Investigator:

Signed: _____ Date: _____

Name:

Title:

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List of Abbreviations

Ab	Antibody
AE	Adverse Event
AIDS	Acquired Immunodeficiency Syndrome
ALT	Alanine Aminotransferase
ASC	Antibody Secreting Cell
AST	Aspartate Transaminase
BM	Memory B cells
CBA	Cytometry Bead Array
CBC	Complete Blood Count
CCR9	C-C Chemokine Receptor Type 9
CD	Cluster of Differentiation
CD3	Pan T-cell Marker
CD4	T-helper Cell
CD8	T-cytotoxic Cell
CD14	Pan-monocyte/macrophage Marker
CD19	Pan-B Cell Marker
CD20	Pan-B Cell Marker
CD25	Interleukin 2 Receptor Alpha Chain
CD27	Tumor Necrosis Factor Receptor Superfamily Member 7
CD38	ASC/plasmablasts/plasma cells Marker
CD45RA	Isoform A of CD45 (Leukocyte common antigen)
CD62L	L-selectin
CD69	Early T-cell Activation Antigen 1
CD103	Integrin Alpha E
CD107	Lysosome-associated Membrane Proteins (LAMP)-1 and LAMP-2
CFR	Code of Federal Regulations
CICERO	Comprehensive Institutional Collaborative Evaluation of Research Online
CMI	Cell-mediated Immunity
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events

CTL	Cytotoxic T Lymphocyte activity
CVD	Center for Vaccine Development
CVD 103-HgR	The strain designation of live oral cholera vaccine, Vaxchora
DHHS	Department of Health and Human Services
DMEM	Dulbecco's Modified Eagle's Medium
DNA	Deoxyribonucleic Acid
EBV	Epstein-Barr virus
e.g.	For Example
EU	European Union
FDA	Food and Drug Administration, DHHS
FOXP3	Forkhead Box P3
FWA	Federalwide Assurance
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
GCP	Good Clinical Practice
GI	Gastrointestinal
HIV	Human Immunodeficiency Virus
HLA-DR	Human Leukocyte Antigen DR
HRPO	Human Research Protections Office
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
IFN-γ	Interferon-γ
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IL	Interleukin
IPC	Internal Position Control
IRB	Institutional Review Board
ISM	Independent Safety Monitor
IV	Intravenous
LCL	Lymphoblastoid Cell Lines
MHC	Major Histocompatibility Complex
MI	Milliliter

MOP	Manual of Procedures
mRNA	Messenger Ribonucleic Acid
NIAID	National Institute of Allergy and Infectious Diseases, NIH, DHHS
NIH	National Institutes of Health, DHHS
OCT	Optimal Cutting Temperature
OHRP	Office for Human Research Protections, DHHS
PBMC	Peripheral Blood Mononuclear Cells
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PWM	Pokeweed Mitogen
QA	Quality Assurance
QC	Quality Control
QMP	Quality Management Plan
RPMI	Roswell Park Memorial Institute medium
rRNA	Ribosomal Ribonucleic Acid
SAC	Staphylococcus aureus Cowan
SAE	Serious Adverse Event
SEB	Staphylococcal enterotoxin B
SMC	Safety Monitoring Committee
SOP	Standard Operating Procedure
TCR	T-cell Receptor
TM	Memory T cells
TEM	Effector Memory T cells
TCM	Central Memory T cells
TEMRA	CD45RA+ Effector Memory T cells
TNF	Tumor Necrosis Factor
Tregs	Regulatory T Cells
UMB	University of Maryland Baltimore
US	United States
WHO	World Health Organization
Yrs	Years

Protocol Summary

Title:	Collection of Blood for In Vitro Studies from Healthy Adults Who Have Received Oral Cholera Vaccine (CVD 103-HgR)
Protocol No.:	Blood Donor CVD 9000
Phase:	Specimen and data collection study
Population:	Total of 200 healthy adults (ages 18 years or older) from the Baltimore-Washington metropolitan area and from the UMB campus
Number of Sites:	1
Study Duration:	indefinite
Volunteer Participation Duration:	Up to 8 years
Agent or Intervention:	Licensed CVD 103-HgR oral *V. cholerae* O1 vaccine (Vaxchora®, PaxVax Inc., Redwood City, CA, USA)
Objectives:	1. To measure systemic and mucosal immune responses following immunization with the oral licensed cholera vaccine in consenting volunteers 2. To measure epigenetic changes which occur in response to vaccination with oral cholera vaccines 3. To integrate these multifaceted datasets into a systems biology approach to the understanding of the response to vaccination

Key Roles

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1 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1.1 Background Information

Vibrio cholerae, the causative agent of cholera, has the ability to cause a rapidly dehydrating diarrheal disease and is also a public health problem of developing countries, travelers, and deployed military personnel to areas lacking safe water and sanitation. A live oral cholera vaccine CVD 103-HgR has been licensed and a single oral dose demonstrated an efficacy of 80-90%.[1] Vaccination with CVD 103-HgR appears to confer sterilizing immunity in the gut.

Despite the availability of such an efficacious vaccine, the development of new and improved vaccines is severely hampered by a lack of information concerning the "true" (i.e., operative) immunological mechanisms underlying the protection elicited by cholera, which does not invade the mucosal surface. Specimens obtained from volunteers participating in this clinical study will be used to identify the immunological mechanisms of protection from *V. cholerae*. These mechanisms may be broadly applied to other enteric pathogens for which vaccines are not yet available.

1.2 Scientific Rationale

Immunological responses to *V. cholerae*

The immunological responses that correlate with protection from *V. cholerae* remain largely undefined or poorly defined. Thus, in depth studies of immune responses will be conducted with specimens from young adults and the elderly immunized with a single dose of CVD 103-HgR in this clinical research study. These studies should provide key insights into the characteristics and longevity of the immune responses that best correlate with protection from infection with *V. cholerae* and contribute to further our knowledge of the mechanisms underlying immunosenescence. Furthermore, these studies will enable a direct comparison of the magnitude and characteristics of the systemic and gut mucosa immune responses elicited by CVD 103-HgR immunization.

Summary

The specimens obtained in this clinical research study will be used to further our understanding of the protective immunological mechanisms that can be elicited systemically and may be applicable to other enteric pathogens.

2 STUDY OBJECTIVES AND OUTCOME MEASURES

2.1 Objectives

1. To measure the systemic immune response following immunization with oral licensed cholera vaccine in consenting volunteers
2. To measure epigenetic change which occur in response to vaccination with oral cholera vaccine
3. To integrate these multifaceted datasets into a systems biology approach to the understanding of the response to vaccination

2.2 Outcome Measures

2.2.1 Primary outcome

Percentage of responders by four-fold increase in vibriocidal antibody titers between pre- and post-immunization time-points.

2.2.2 Exploratory outcomes

The following brief descriptions provide some overview of the intended laboratory analyses to be performed with the specimens to be collected in this study. *This is not meant to be an exhaustive list of all the possible laboratory studies to be performed*.

1. Functional analysis of systemic (e.g., using serum, PBMC) anti-*V. cholerae* immune responses. These studies will include the following measurements, depending on specimen availability:
    a. Ab levels to *V. cholerae* antigens in bodily fluids (e.g., serum, plasma)
    b. Ab-secreting cells (ASC) to *V. cholerae* antigens
    c. Cytokine production (e.g., intracellular IFN-γ, TNF-α, etc. by multichromatic flow cytometry or mass cytometry, IFN-γ production by Elispot and/or cytokine production in culture supernatants by multiplex technology.)
    d. Memory (T_M) and B_M responses
    e. CTL
    f. innate immune responses (macrophages and dendritic cells)
2. Evaluation of homing characteristics of the various *V. cholerae*-responsive CD4+ and CD8+ T cell subsets
6. Study of TCRγδ T cells and T_{reg} using multichromatic flow cytometry or mass cytometry using monoclonal antibodies to molecules that are characteristic of these cells (e.g., CD25 and FOXP3)
7. Measure the expression of host response genes to vaccination using functional genomics,

proteomics, and epigenetics

3 STUDY DESIGN

This study is a single center, open label clinical research study to study the effect of oral immunization with the CVD 103-HgR cholera vaccine in younger adults and older adults (i.e., elderly). Healthy adults, ages 18 years to 64 years, will be recruited from the Baltimore-Washington area in response to campus flyers, advertising, and word-of-mouth. Study participants will receive the cholera vaccine. Subsequent to vaccination, small volume (up to 200 mL) or large volume (200 mL or greater) blood draws will be requested; the timing of these blood draws are not strictly set and the schedules in *Appendix D* and *E* are provided to give a general overview of the flow of research procedures. The timing of the blood draws and the types (small or large volume blood) is dependent on the research laboratory's needs. In general, more frequent scheduled blood draws will occur in the first year after vaccination and less often in the years after vaccination (up to 8 years of planned follow-up). Therefore, we intend to regularly re-screen for anemia and have set limits on the total volume of blood to be collected over any 56 day period.

3.1 Screening

After informed consent has been completed, a medical history, concomitant medications, and vital signs are collected. Initial screening labs for eligibility include:

- Complete blood count (CBC) with differential, to assess total white blood count (WBC), hemoglobin, and platelet count
- Liver function tests to assess alanine aminotransferase (ALT) and aspartate aminotransferase (AST)
- Serologies for HIV
- Blood typing (this is not an eligibility criterion, but is used to guide the immunological analysis)

3.2 Interventions

Administration of licensed Live Oral Cholera Vaccine CVD 103-HgR. Participants will be given the vaccine, as packaged, and are to administer the vaccine as per the package insert. Briefly, approximately 100 mL of cool or room temperature purified bottled water is to be placed into a clean disposable cup. The contents of the buffer sachet are to be placed into the water and stirred. Then the contents of the active (lyophilized vaccine) sachet are to be placed into the water, then stirred for ~30 seconds. The reconstituted mixture should be completely ingested within 15 minutes. Illustrated instructions for the administration of CVD 103-HgR is presented in *Appendix E.*

Serial Blood Donor (small volume). After volunteers have been recruited, they may be contacted to have blood drawn, 1-20 times a year. No more than 2 ⅓ cups (550 ml) will be taken over a 2-month period of time. The volunteer will be given a schedule of the dates when blood will be drawn and the amount of blood required each time they are contacted. At any time a donor may decline a blood specimen but choose to remain in the study. In most instances, 20 to 200 mL (4 to 40 teaspoons) will be collected at one visit. Visits for one-time or serial blood donations will take approximately ten minutes each and are performed at the Center for Vaccine

Development (CVD) Outpatient Research Clinic.

Blood Unit Donor (large volume). When large volumes of blood are desired, participants may be directed to the Greenebaum Cancer Center (GCC) Cell Component Therapy (CCT) Lab. Visits for the donation of a unit of blood/large volume (200- 500 mL) will take one hour. As part of the GCC CCT lab's standard operating procedure, a CBC will be performed on the day of donation (prior to donation of a unit of blood) for verification of a hemoglobin 12.5 or greater. If the hemoglobin is less than 12.5, the volunteer will be deferred and re-screened at a later date or they may have a smaller volume blood draw (≤ 200 mL) performed at the CVD Outpatient Research Clinic, if eligible by the hemoglobin criteria in *Appendix C.* Additional blood draws will take place according to the schedules attached.

Planned repeat screening labs. The screening blood tests (except for blood typing) will be repeated prior to each scheduled blood donation (small or large volume), but not more often than every 56 days. This is primarily to ensure that the participant is not significantly anemic, but also assesses for any interval abnormality in blood tests since the study duration may be over the course of 8 years.

3.3 Potential Risks

CVD 103-HgR Vaccination

The live oral cholera vaccine, known as Vaxchora (composed of strain CVD 103-HgR), was licensed by the U.S. FDA on June 10, 2016. The adverse reactions to CVD 103-HgR vaccine are generally mild and may include tiredness (30%), headache (28%), abdominal pain (18%), nausea/vomiting (17%), lack of appetite (16%), and diarrhea (4%). The safety and immunogenicity of the live oral cholera vaccine has not been fully established in children (age <18 years), pregnant women, or immunocompromised patients. However, a phase 2 study of the immune response to CVD 103-HgR was performed in HIV patients.[2]

Risk with Venipuncture

Venipuncture is associated with temporary local pain, some bruising, light-headedness or fainting, and a risk for infection. Infection risk is minimized by the use of alcohol swabs and sterile equipment. Blood will be drawn by trained personnel using clean technique. Volunteers who are found to experience lightheadedness will lie down during and after they have their blood drawn.

Risk of Anemia

Because serial blood draws will be performed in this protocol, there is a risk of anemia. The volumes of blood drawn from each volunteer will be limited to 550 mL every 56 days, and the hematocrit will be checked at regular intervals. Participants found to be anemic will have their scheduled blood draws postponed, be advised to increase iron intake, and have a hematocrit repeated and in the normal range in order to be eligible for the blood draw.

Risk of Loss of Confidentiality

There is always the risk for loss of confidentiality when participants are sharing their personal information. As a standard procedure for all clinical studies, access to clinical information will be limited to the IRB-approved clinical investigators, study coordinator, and research staff. Paper source documents and Case Report Forms (CRFs) will be stored in limited-access rooms and

locked file cabinets. Electronic CRFs will be password protected in a limited access database. IRB-approved research staff will be the only individuals with access to the personal identifiable information and personal health information of research participants.

3.4 Known Potential Benefits

Vaccine against *V. cholerae* is intended to protect the immunized in endemic areas, as well as travelers (including military personnel) from industrialized countries to regions endemic for *V. cholerae*, and in the event of a threat or exposure to a biological weapon. There is no known benefit to receipt of off-label use of the CVD 103-HgR vaccine.

V. cholerae is a global pathogen that can disseminate rapidly in settings where there is crowding and inadequate sanitation. The symptoms of cholera are predominantly a rapidly dehydrating diarrheal disease without significant systemic toxicity.

This clinical research study is intended to greatly further our understanding of the protective immunological mechanisms that can be elicited systemically. This information is intended to inform the development of newer generation cholera vaccines.

3.5 Risk/Benefit Ratio

Alternative approaches are limited for these studies. The risks of the study interventions are low. The CVD 103-HgR vaccine has been extensively used, and their side effect profiles are tolerable and well-understood. Venipuncture also carries a low risk when conducted by a trained phlebotomist. On the other hand, enteric infections are widespread throughout the globe and are associated with significant morbidity and mortality. The complexity of the immune response after vaccination cannot be reproduced in other environments. The risks associated with the procedures described in this protocol are small compared to the potential general benefit of better understanding the protective immunity elicited by these vaccines.

3.6 Specimen Management

All specimens collected through this protocol will be labelled with the volunteer identification number and date, but not any volunteer identifiable information (such as date of birth). Specimens that are processed for storage will also be labelled in a manner which is de-identified and contains no identifiable information. Specimens are entered into specimen tracking form to link them with the volunteer identification number. Only the clinical PIs and study coordinator(s) will have access to the linking information. Specimen handling, processing, and storage will be according to internal CVD standard operating procedures.

4 STUDY ENROLLMENT AND WITHDRAWAL

The study enrollment will include adults from the local area. The study will not exclude any race, ethnicity or gender from enrollment. Women of child bearing potential will have a urine pregnancy test prior to vaccination. Women testing positive will be excluded from the study. Volunteers will have frequent contact with the study coordinator(s) to facilitate retention and complete data collection. The inclusion/exclusion criteria will be reviewed with each volunteer during the consent process. The clinical principal investigator or designee is responsible for reviewing and signing off the inclusion and exclusion criteria for all enrollees.

4.1 Informed Consent

All aspects of the protocol will be explained in depth to volunteers including the specific purpose of the blood draws. Volunteers will be given a schedule of when, where, and how much blood will be drawn. The consent form will be read by the volunteer and ample time for questions and answers will be provided. Interested volunteers will provide written consent prior to any procedures being performed. Volunteers will remain enrolled as a blood donor as long as the laboratory has need for the blood (which may continue for several years), or until the volunteer withdraws consent. The informed consent process and other health assessment questions will take place in a private or semi-private room. Privacy and confidentiality of the volunteer will be maintained.

4.2 Inclusion Criteria

Volunteers must meet all inclusion criteria in order to be eligible to participate.

1. Age 18 years to 64 years at the time of enrollment
2. Good general health as determined by a screening evaluation within 28 days before blood donation or vaccination
3. Provides written informed consent prior to initiation of any study procedures

4.3 Exclusion Criteria

Volunteers must meet no exclusion criteria in order to be eligible to participate

1. History of any of the following medical conditions:
    - Diabetes
    - Cancer in past 5 years (except for basal cell carcinoma of the skin and cervical carcinoma in situ)
    - Heart disease (e.g., hospitalization for a heart attack, coronary artery bypass graft, arrhythmia, or syncope, within past 5 years. Current symptoms of heart disease, such as dyspnea, angina, or orthopnea)
    - Recurrent infections (e.g., more than 3 hospitalizations for invasive bacterial

infections such as pneumonia or meningitis)
- Current drug or alcohol abuse
- Active ulcer disease or ongoing intestinal condition
- Treatment for anemia in last 6 months
- Treatment with anti-malarial drugs within ten days prior to study vaccination
- Treatment with antibiotics within 14 days prior to study vaccination
- Immunodeficiency or immunosuppression from illness or treatment

2. Close contact within 7 days following study vaccination with a person who has an immunodeficiency or immunosuppression from illness or treatment
3. History of cholera infection or cholera vaccination
4. Any of the following CBC abnormalities during screening:
    - WBC <0.81 x LLN or > 1.09 x ULN
    - Hemoglobin <0.91 x LLN or >1.18 x ULN (women) or <0.92 x LLN or >1.18 x ULN (men)
    - Platelet count <0.8 x LLN or > 1.2 x ULN
5. Any of the following laboratory abnormalities during screening:
    - SGOT or SGPT >1.5 times normal
    - Positive serology for HIV antibody
6. Poor peripheral venous access for blood donation
7. Other condition that the opinion of the investigator would jeopardize the safety or rights of a volunteer participating in the trial or interfere with the scientific integrity of study
8. Positive urine pregnancy test (HCG) on the day of vaccination

4.4 Delayed Vaccination

Study participants that have an acute illness, fever, or gastrointestinal complaint (temporary) within the 3 days prior to scheduled vaccination should have their vaccination delayed until recovery from their temporary illness. Study participants that are on a temporary course of concomitant antibiotics should have their vaccination delayed until at least 7 days after completion of antibiotics. Study participants on certain potentially interfering malaria prophylaxis medications (chloroquine), should have their vaccination delayed—dosing may begin 2 weeks after completion of chloroquine.

4.5 Withdrawal

A study volunteer will be discontinued from study participation for the following reasons:
- Request by volunteer to terminate participation
- Requirement for prohibited concomitant medication or treatment
- Unable to comply with requirements of the protocol
- Failure to comply with study procedures
- At the request of the IRB or NIAID
- The volunteer's well-being, based on the opinion of the investigator

The PI may remove volunteers from the study if they develop adverse events (AEs) that are believed to be related to the study or unrelated but may impact the safety of the volunteer or the integrity of the study. These might include abnormal laboratory values or test procedure results, medical treatments that might interfere with immune responses or use of other investigational products or vaccines during the study period. Development of exclusion criteria may also prompt exclusion from the study. Volunteers may also be withdrawn for failure to comply with study procedures. Volunteers may withdraw consent at any time upon request.

4.6 Termination of the Study

This study may be closed by the NIH, the principal investigator, or the UMB IRB. If the study is terminated prematurely the study participants will be notified in writing. A final safety evaluation will be done for all volunteers enrolled at the termination of the study.

5 INVESTIGATIONAL PRODUCT(S)

5.1 Cholera Vaccine (CVD 103-HgR)

5.1.1 Formulation, Packaging, and Labeling

CVD 103-HgR, marketed as Vaxchora®, is provided as two powder sachets, containing buffer and lyophilized organisms, in a single package.

5.1.2 Acquisition

Product will be obtained from the manufacturer PaxVax, Inc (Redwood City, CA, U.S.A.)

5.1.3 Product Storage, Stability, and Expiration

Vaxchora (Cholera Vaccine, Live, Oral) is to be stored at -15° C or colder (5° F or colder). Each package of vaccine shows an expiration date. This expiration date is valid only if the product has been maintained at -15° C or colder.

5.1.4 Preparation/Handling/Administration

Vaxchora (Cholera Vaccine, Live, Oral; CVD 103-HgR) is indicated for immunization of adults 18-64 years of age against disease caused by *V. cholerae*. [*NOTE: Vaxchora is currently not licensed for older adults (age 65 years or older) and thus older adults are not intended to be vaccinated with Vaxchora in this study.*] Vaccine potency is dependent upon storage under freezing (-15° C or colder; 5° F or colder). The vaccine should be reconstituted and ingested within 15 minutes of removing from the freezer. Recipients should avoid eating or drinking for 60 minutes before and after ingestion of Vaxchora.

Participant will take a single oral dose of the CVD 103-HgR vaccine. A volume of ~100 mL of purified bottled water (at cold or room temperature) shall be placed into a clean disposable cup. The buffer component sachet is emptied into the cup and stirred. Then, the active vaccine (lyophilized *V. cholerae* CVD 103-HgR) component is emptied into the cup and stirred, for ~30 seconds. The mixed contents should be completely consumed within 15 minutes of removal from the freezer.

5.2 Maintaining Blind for Investigational Product(s)

This study is not blinded.

5.3 Accountability/Final Disposition for the Investigational Product(s)

Vaccine will be kept in a secure area and stored according to package directions. The CVD will maintain an accurate record of shipping and dispensing of investigational products in an accountability ledger. The ledger will include the date and amount of investigational product dispensed to each volunteer.

6 MANAGEMENT OF SAFETY

6.1 Specification of Safety Parameters

The clinical investigators are responsible for reporting Adverse Events (AEs) that are observed or reported during the study. Serious Adverse Events (SAEs) must be reported as soon as the clinical-investigators become aware. See Section 7.2 for reporting windows. Only AEs and SAEs occurring within 7 days post vaccination determined to be related to the study product or 1 day post blood donation will be collected. No AE or SAE will be collected outside the window mentioned above.

6.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

Adverse Event (AE)

ICH E6 defines an AE as any untoward medical occurrence in a patient or clinical investigation volunteer administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews of a study recipient presenting for medical care, or upon review by a study monitor.

All AEs including local and systemic reactions not meeting the criteria for “serious adverse events” should be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician’s assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis, which would include MD, PA, Nurse Practitioner, DO, or DDS), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately, AEs occurring within 7 days post vaccination determined to be related to the study product and/or 1 day post-blood donation and determined to be related to the procedure will be collected. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the patient is screened should be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study, it should be recorded as an AE.

All AEs must be graded for severity and relationship to study product. FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Severity of Event: The following guidelines will be used to quantify intensity.

Mild: events require minimal or no treatment and do not interfere with the patient’s daily activities.

Moderate: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

Severe: events interrupt a patient’s usual daily activity and may require systemic drug therapy

or other treatment. Severe events are usually incapacitating.

Life threatening: any adverse drug experience that places the patient or volunteer, in the view of the investigator, at immediate risk of death from the reaction as it occurred, ie, it does not include a reaction that had it occurred in a more severe form, might have caused death.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

Relationship to Study Products: The clinician's assessment of an AE's relationship to vaccine is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study product assessed using the terms: related or not related. In a clinical study, the study product must always be suspect. To help assess, the following guidelines are used.

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

Serious Adverse Event (SAE)

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

- death,
- a life-threatening adverse event*,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or
- a congenital anomaly/birth defect.
- important medical events that may not result in death, be life-threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment they may jeopardize the patient or volunteer and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

* *Life-threatening adverse event. An adverse event is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or volunteer at immediate risk of death. It does not include an adverse event, had it occurred in a more severe form, might have caused death.*

All SAEs will be:

- recorded on the appropriate SAE CRF
- followed through resolution by a study clinician
- reviewed and evaluated by a study clinician

The SAEs must be reported as soon as the investigator becomes aware but within 5 working days for internal events, consistent with UMB policy Investigators will report the SAE using the Adverse Event form in the Comprehensive Institutional Collaborative Evaluation of Research Online (CICERO) electronic system. The investigator will provide a summary of the problem or event in the text box labeled “adverse event information”. The summary should include type or nature of the problem, a full description of the activities leading to the problem, interventions and/or actions taken in response, temporal relationship of event to the use of the investigational product/procedure and the current status of the participant. Additional information will be reported as it is received.

In the event of any serious adverse experience which, in the clinical investigator’s opinion, justifies termination or modification of the study, it will be stopped and the IRB and ISM will be informed immediately. The IRB will be notified via CICERO, the IRB electronic tracking system.

Reactogenicity

Reactogenicity will not be measured in this study.

6.3 Reporting Procedures

AEs will be followed until resolution. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic.

AEs and SAEs will be received, reviewed and managed by the ISM. The investigator is responsible for reporting events to VAERS according to the VAERS reporting criteria provided in the federal regulations. Regulatory Reporting of AEs that are determined to be vaccine related will be reported through the Vaccine Adverse Event Reporting System (VAERS).

6.4 Other Adverse Events (if applicable)

An unexpected AE is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., package insert for the respective vaccine).

6.5 Reporting of Pregnancy

Although not AEs, pregnancies are reportable events (on the study-specific form) to the data management group. The pregnancy outcome will be reported if known including the health status of the mother and child including date of delivery and infant’s gender and weight. If a volunteer’s urine pregnancy test is positive prior to receiving the vaccination, they will be excluded from participating in the study.

Volunteers who are pregnant prior to receiving the vaccination will be excluded from participation in this study. Pregnancy will be reported as an unanticipated problem involving risk to research participants. Pregnancy will be reported to the UMB IRB within 5 working days of the time the research staff become aware of the pregnancy. In the event a volunteer becomes pregnant after

taking the vaccine the volunteer will be encouraged to complete all visits for safety follow up.

6.6 Type and Duration of Follow-up of Volunteers after Adverse Events

All SAEs will be followed until resolution even if this extends beyond the study-reporting period. Resolution of an AE is defined as the return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic. The Clinical-PIs will follow all SAEs until resolution (return to pretreatment status or stabilization of the condition with the expectation that it will remain chronic) even if this extends beyond the study-reporting period.

At any time after completion of the study, if the investigator becomes aware of an SAE that is suspected to be related to study product, the investigator will report the event to the IRB.

6.7 Independent Safety Monitor

Dr. Monica A. McArthur will be the ISM for this clinical research study.

6.8 Oversight of Safety

The clinical investigators reserve the right to terminate this study should serious AEs or any other safety issue occur during the study. The UMB IRB upholds that all currently approved research that is not being conducted in accordance with the Human Research Protections Program and UMB IRB policies and procedures, Federal, State, and local requirements, or has been associated with unexpected serious harm to participants may be subject to suspension or termination. The IRB has the authority to suspend or terminate approval of research not being conducted in accordance with the IRB's requirements or that had been associated with unexpected serious harm to participants.

6.9 Clinical Monitoring/Site Monitoring Plan

The study will be monitored by the site clinical PIs. On a regular basis the clinical PIs will review patient charts/clinical reports, laboratory tests, outcomes, enrollment, procedure reports, and all adverse events. Site monitoring is conducted to ensure the human subject protection, study procedures, laboratory, study intervention administration and data collection processes are of high quality and meet sponsor, ICH E6, and other appropriate, regulatory guidelines and that the study is conducted in accordance with the protocol.

7 STATISTICAL CONSIDERATIONS

7.1 Study Hypothesis

This is an exploratory study rather than a hypothesis-driven study.

7.2 Sample Size Considerations

This study is exploratory in nature and is designed to identify associations among the presence and quantity of various intestinal bacteria, immune responses and clinical outcome. Thus it is designed to generate hypotheses but not to test any specific hypothesis based on prior data since no such data is available in the literature. The sample sizes planned for the study are therefore based on feasibility rather than power to reject any specific hypothesis. For large differences between groups the study will have good power to find statistically significant associations.

Data for withdrawn volunteers will be included in the intent to treat group. We anticipate a dropout rate of 20%. This is consistent with other studies of this nature performed at the University of Maryland.

7.3 Final Analysis Plan

The statistical analysis will depend on the hypothesis being evaluated. For example, if measuring cytokine levels, the level of cytokine, in pg/ml, will be obtained from extrapolation over standard curves. Seroconversion following challenge will be defined as a fourfold rise in serum Ab over pre-challenge levels. The geometric mean Ab level will be calculated for each time point and the peak time determined. The proportion of volunteers in each group who seroconvert will be determined for each antigen and Ab class at the peak time post-challenge. A positive ASC response will be defined for each immunoglobulin class and antigen as a post-vaccination count of at least 8 ASC/10^6 PBMC that is ≥ 3 standard deviations above the mean pre-vaccination count for all volunteers in all cohorts combined. The proportion of volunteers in each group who develop each specific ASC response will be determined for each antigen and Ab class measured. The geometric mean ASC level at each time point and the peak post-immunization geometric mean will be calculated. Comparisons between systemic and local responses will be performed by using a two-sample t-test on log10 values or a Wilcoxon rank-sum test.

Comparisons between pre- and post-immunization levels within a single group will use a one-sample t-test on log10-transformed values or a Wilcoxon signed-rank test. Comparisons between two groups will use a two-sample t-test on log10 values or a Wilcoxon rank-sum test or the Kolmogorov-Smirnov test. Comparisons of proportions responding will use chi-square or exact tests. For comparisons among three or more groups, an overall analysis of variance or Kruskal-Wallis test will be done first; if that test indicates significant heterogeneity among groups, pairwise comparisons will then be made, with adjustment for the number of comparisons. Continuous immunological responses will be compared in the young adults and elderly by analysis of variance or Kruskal-Wallis test and pairwise comparisons as appropriate. Proportions of responders will be compared by chi-square or exact tests. Relationships between immunological measurements will be assessed using correlation analyses (for two continuous responses), t-tests (for comparing a continuous and a dichotomous response), and chi-square tests or exact tests (for comparing

two dichotomous responses). Two-sided p-values will be reported whenever two groups, or pre- and post-immunization levels within a group, are compared. $P < 0.05$ will be considered statistically significant. No imputation will be made for missing data.

8 DATA HANDLING/RECORD KEEPING/SOURCE DOCUMENTS

The investigators are responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. Data will be recorded on source documents and CRFs in a neat, legible, and timely manner using black ink to ensure accurate interpretation of data and clarity of reproduced copies. When making a change or correction, the original entry will be crossed out with a single line. The initials of the person making the change and the date of the change will be recorded next to the change. Corrections will NOT be made by erasing, overwriting, or using correction fluid or tape on the original. Dates reported in the CRF derived from source documents will be consistent with the source documents or the discrepancies will be explained. After data are quality controlled by the clinical or laboratory staff, they will be entered into a database and checked for accuracy, consistency and completeness, according to data entry standard operating procedures (SOPs).

The Principal and Co-Principal investigators assume responsibility for the accuracy and security of study data obtained, managed, stored, and reported. Data collection is the responsibility of trained study clinical and laboratory staff under the supervision of the project leader. Data management core facilities such as the University of Maryland School of Medicine Bioinformatics Core Facility may be responsible for data management including data capture and quality review. Adverse events must be graded, assessed for severity and causality, and reviewed by the site PI or designee.

All data collection and analysis will be performed using coded samples. Coding will occur at sample collection. Investigators will remain blinded to volunteer identifiers during data review and analysis.

8.1 Data Capture Methods

Clinical data (including AEs, concomitant medications, and clinical laboratory data) will be entered into paper record clinical research forms. The clinical research forms will remain the authoritative record.

Data capture from these source documents will be stored in a password-protected electronic database managed by REDCap, a 21 CFR Part 11-compliant CRF and database. The data system includes password protection and internal quality checks, such as automatic range checks to identify data that appear inconsistent, incomplete, or inaccurate. In some cases the CRF will serve as a source document.

Source documents will be reviewed by study staff receiving the source document (as in clinical team receiving the screening laboratory report) or by study staff generating the data (as in and data entry staff, who will ensure that they are accurate and complete). Adverse events will be graded, assessed for severity and causality, and reviewed by the Clinical Investigator or designee

8.2 Types of Data

Clinical, immunological, microbiological, and other laboratory data may be generated as part of the projects. Clinical data may be obtained from clinical source documents or directly from the participant as documented by trained clinical research staff. Immunological, microbiological, and

other data will be generated by laboratory staff. Source documents are the documents on which data collected is first recorded.

Safety data are collected on CRFs in the patient study record and recorded aggregately in a separate study log.

8.3 Study Records Retention

Records for the study will be retained for 5 years as required by University of Maryland Human Research Protections Office policy.

The site will maintain appropriate medical and research records for this study, in compliance with ICH E6 and regulatory and institutional requirements for the protection of confidentiality of volunteers. The site participating in this study will permit authorized representatives of the sponsor and regulatory agencies to examine (and when required by applicable law, copy) clinical records for the purposes of clinical site monitoring, quality assurance reviews, audits, and evaluation of the study safety and progress.

8.4 Source Documents

The clinical history recorded in the volunteer's medical records will provide source data for participants in this study. This data is captured both electronically and in paper copy. Clinical laboratory data are reported electronically. A paper printout of volunteer data will provide the source document for laboratory data that is generated electronically either in the clinical or research laboratory setting, whenever possible. Data capture from source documents will be collected into a database managed by REDCap. The data, as entered on the forms, constitutes the "official" study data and any computerized data is required to be in agreement with the information as recorded on the paper forms.

8.5 Protocol Deviations

A protocol deviation is any noncompliance with the clinical study protocol or GCP. The noncompliance may be either on the part of the volunteer, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

UMB IRB will be notified within 5 working days of all deviations and violations that result in an increase in risk or a decrease in benefit to participants and all unanticipated problems involving risk to research participants and others. Deviations that do not alter the risk benefit analysis or integrity of the study can be submitted at the time of annual renewal or in an expedited fashion if the sponsor requires such reporting. All deviations from the protocol must be addressed in study volunteer source documents. Protocol deviation reporting will be according to CVD standard operating procedures.

8.6 Quality Control and Quality Assurance

Internal audits serve to verify that the clinical study/research is conducted according to the IRB-

approved protocol and in compliance with the International Conference on Harmonization (ICH) and Good Clinical Practice Guideline (GCP), applicable U. S. Code of Federal Regulations (CFR) Titles 21, Parts, 50, 54, 56, 58 and 312, US FDA Guidances, Department of Health and Human Services (DHHS) Title 45 part 46, and University of Maryland Human Research Protections Office (HRPO) polices.

A Quality Management Plan developed for this protocol will identify the protocol, state the frequency of quality control checks, quality assurance audits, priority of QA reviews, and the percentage of charts that will be selected for QA review. The plan will describe how data will be evaluated for compliance with the protocol, completeness, and accuracy.

Processes to be reviewed include, but are not limited to: eligibility, informed consent process and documentation, concomitant medication recordings, study product accountability and management, AE/SAE reporting, study/clinical endpoints, follow-up visits, missed visits, study discontinuation, and review of regulatory documents.

The QMP and written quality assurance communication (e.g., audit reports, problem resolution worksheets, regulatory checklists, audit summaries, QC/QA trend reports, quarterly and annual reports, etc.) for this study will be kept in a separate quality management file.

9 ETHICS/PROTECTION OF HUMAN SUBJECTS

9.1 Ethical Standard

The investigator(s) will ensure that this study 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 312, and/or ICH E6; 62 Federal Regulations 25691 (1997). The PI/Institution will hold a current federal wide assurance (FWA) issued by Office of Human Research Protections, DHHS (OHRP) for federally funded research

Volunteers will provide informed consent prior to any participation in the study. Volunteers may withdraw consent at any point in the study. Specimens may not be used for future research without the volunteer's written permission. Pregnant women will be excluded from the research because of the potential for increased risk from live vaccines. No other special population will be excluded.

9.2 Institutional Review Board

A copy of the protocol, informed consent forms (ICF), and other information to be completed by participants, such as survey instruments or questionnaires, and any proposed advertising / recruitment materials will be submitted to the IRB for written approval. The investigator must submit and obtain approval from the IRB for all subsequent amendments to the protocol, informed consent documents and other study documentation referenced above. The investigator will be responsible for obtaining IRB approval of the annual continuing review throughout the duration of the study. The investigator will notify the IRB of violations from the protocol and serious adverse events.

9.3 Informed Consent Process

The informed consent process will be initiated before a volunteer agrees to participate in the study and should continue throughout the individual's study participation. The consent will explain that volunteers may withdraw consent at any time throughout the course of the study. Extensive explanation and discussion of risks and possible benefits of this investigation will be provided to the volunteers in understandable language. Adequate time will be provided to ensure that the volunteer has time to consider and discuss participation in the protocol.

The consent forms will describe in detail the study interventions/products/procedures and risks/benefits associated with participation in the study. The rights and welfare of the volunteers will be protected by emphasizing that their access to and the quality of medical care will not be adversely affected if they decline to participate in this study.

The volunteer will sign the informed consent document before any procedures are undertaken for the study. A copy of the signed informed consent document will be given to the volunteer for his/her records.

9.4 Volunteer Confidentiality

Volunteer confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participating volunteers. All electronic records will be password protected in a limited access database. Paper records will be stored in a locked cabinet with access limited to persons involved in the research. Only the PIs and staff will have access to the data. Specimens will be identified by barcode linked to volunteer ID. The table linking the volunteer ID to the volunteer's identifying information will be maintained in a password-protected network file, with access only by the clinical PIs and study coordinator(s). Any published data will have all identifiers removed.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

The study monitor or other authorized representatives of the sponsor may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the volunteers in this study. The clinical study site will permit access to such records.

Records for the study will be retained for 5 years as required by University of Maryland policy.

Volunteer confidentiality will be protected to the fullest extent permitted by law. Volunteer names will not be used in any published report. Study records may be confidentially reviewed by federal agencies such as National Institutes of Health (NIH), and the IRB.

9.5 Principal Investigator Responsibility When Volunteer Withdraws or is Discontinued

In the event that the study is discontinued volunteers will continue to receive standard clinical care.

9.6 Future Use of Stored Specimens (if applicable)

Volunteers will indicate if specimens may be used for future research as part of the consent form. Specimens will be coded and banked in the CVD specimen bank. Any future studies will be reviewed by the UMB IRB prior to specimen use. Genetic testing may not be conducted on these specimens without the approval of the IRB. Future genetic testing might include the presence of genes or particular haplotypes or variants that might impact either positively or negatively the host immune response to vaccination. No genetic testing for hereditary diseases will be done. We do not anticipate that all of the samples obtained as part of this research study will be used during the course of the study. Remaining samples will not be discarded, but instead will be frozen and kept indefinitely for future CVD 103-HgR research. Banked samples will not be made available to the research volunteer (or his/her medical doctor) for other testing. If a participant withdraws from the study they will not have the option to get the remaining portion of their sample(s) back; however, the sample(s) and all data for that participant will be anonymized with a unique identification code. This code will not be linked or identifiable to a specific participant. Volunteers

who withdraw from the study and decline in the consent form to have specimens used for future research will have specimens discarded.

If specimens have to be shipped to other institutions for specialized measurements (e.g., functional genomics, proteomics), we will seek prior approval of the UMB IRB to enable the shipment of the specimens as well as approval of the recipient institution's IRB to enable the receipt and processing of such specimens. Only coded specimens will be shipped. Under no circumstances will information linking the code in the specimens to the name of the volunteer be made available to investigators receiving these specimens at other institutions.

10 PUBLICATION POLICY

If the data merit, the investigators will prepare one or more manuscripts for publication in peer-reviewed professional journals or abstracts for presentation, oral or written, to a learned society or symposium. All manuscripts will contain appropriate acknowledgement of the funding mechanism.

The PI is responsible for the timely submission of all abstracts, manuscripts and reviews (co)authored by investigators and supported in part or in total by this protocol. The PI and project leaders are requested to submit manuscripts to the Program Officer within two weeks of acceptance for publication so that an up-to-date summary of program accomplishments can be maintained and joint press conferences and press releases prepared. Publications or oral presentations of work performed under this protocol are the responsibility of the PI and appropriate project leaders and will require appropriate acknowledgement of NIAID support.

All investigators will submit or have submitted for them to the National Library of Medicine's PubMed Central an electronic version of their final, peer-reviewed manuscripts upon acceptance for publication, to be made publicly available no later than 12 months after the official date of publication. The NIH Public Access Policy ensures the public has access to the published results of NIH-funded research. It requires investigators to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication. Further, the policy stipulates that these papers must be accessible to the public on PubMed Central no later than 12 months after publication.

11 LITERATURE REFERENCES

1. Chen WH, Cohen MB, Kirkpatrick BD, Brady RC, Galloway D, Gurwith M, Hall RH, Kessler RA, Lock M, Haney D, Lyon CE, Pasetti MF, Simon JK, Szabo F, Tennant S, Levine MM. Single-dose Live Oral Cholera Vaccine CVD 103-HgR Protects Against Human Experimental Infection With Vibrio cholerae O1 El Tor. Clin Infect Dis. 2016;62(11):1329-35. doi: 10.1093/cid/ciw145. PubMed PMID: 27001804; PMCID: PMC4872293.
2. Perry RT, Plowe CV, Koumare B, Bougoudogo F, Kotloff KL, Losonsky GA, Wasserman SS, Levine MM. A single dose of live oral cholera vaccine CVD 103-HgR is safe and immunogenic in HIV-infected and HIV-noninfected adults in Mali. Bull World Health Organ. 1998;76(1):63-71. PubMed PMID: 9615498; PMCID: PMC2305629.

Appendix A. Typical Study Schedule: Serial Blood Donors[3]

Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Day	**-7 to -28**	**1**	**3 ±1**	**6 ±1**	**8 ±1**	**29 ±2**	**91 ±3**	**181 ±5**	**361 ±30**	**721 ±3m**	**4 Yr ±3m**	**5 Yr ±3m**	**6 Yr ±3m**
Informed Consent obtained	X												
Inclusion/exclusion Review	X												
Urine Pregnancy test		X											
Interim History		X	X	X	X	X	X	X	X	X	X	X	X
Vaccinate with **C**VD 103-HgR		C											
Screening laboratories[1]	X 15mL						X 15 mL	X 15 mL	X 15 mL	X 15 mL	X 15 mL	X 15 mL	X 15 mL
CBC w/differential[2]		X 4mL	X 4mL	X 4mL	X 4mL	X 4mL	X 4mL	X 4mL	X 4mL	X 4mL	X 4mL	X 4mL	X 4mL
PBMC		X 100 mL	X 100 mL	X 85 mL	X 85 mL	X 85 mL	X 100 mL	X 100 mL	X 100 mL	X 100 mL	X 100 mL	X 100 mL	X 100 mL
Serum		X 8.5 mL	X 8.5 mL	X 8.5 mL	X 8.5 mL	X 8.5 mL	X 8.5 mL	X 8.5 mL	X 8.5 mL	X 8.5 mL	X 8.5 mL	X 8.5 mL	X 8.5 mL
Gene expression		X 3mL	X 3mL	X 3mL	X 3mL	X 3mL	X 3mL	X 3mL	X 3mL	X 3mL	X 3mL	X 3mL	X 3mL

1 – Screening laboratory can be performed up to 28 days prior to the scheduled clinic visit and includes: CBC with differential, AST, ALT, and HIV antibody. The screening laboratory results must be reviewed prior to the scheduled blood draw for the respective visit.

2 – CBC w/differential is to be performed on the scheduled clinic visit, with the blood draw for PBMC. It is intended for the back-calculation of the number and concentration of lymphocyte subsets.

3 – Not all volunteers will follow the same study schedule. The schedule will vary according to laboratory needs. No more than 550 mL of blood will be drawn within a 56 day period.

Appendix B. Typical Study Schedule: Large Volume/unit Blood Donors[3]

Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**
Day	**-7 to -28**	**1**	**3 ±1**	**8 ±1**	**29 ±5**	**91 ±7**	**181 ±30**	**361 ±30**	**721 ±3m**	**4 Yr ±3m**	**5 Yr ±3m**	**6 Yr ±3m**	**8 Yr ±3m**
Informed Consent obtained	X												
Inclusion/exclusion Review	X												
Urine Pregnancy test		X											
Interim History		X	X	X	X	X	X	X	X	X	X	X	X
Vaccinate with **C**VD 103-HgR		C											
Screening laboratories[1]	X 15mL					X 15 mL	X 15 mL	X 15 mL	X 15 mL	X 15 mL	X 15 mL	X 15 mL	X 15 mL
CBC w/differential[2]		X 4mL	X 4mL	X 4mL	X 4mL	X 4mL	X 4mL	X 4mL	X 4mL	X 4mL	X 4mL	X 4mL	X 4mL
PBMC		X 150 mL	X 150 mL	X 100 mL	X 100 mL	X 200 mL	X 200 mL	X 200 mL	X 200 mL	X 200 mL	X 200 mL	X 200 mL	X 200 mL
Serum		X 8.5 mL				X 8.5 mL	X 8.5 mL	X 8.5 mL	X 8.5 mL	X 8.5 mL	X 8.5 mL	X 8.5 mL	X 8.5 mL
Gene expression		X 3mL	X 3mL	X 3mL	X 3mL	X 3mL	X 3mL	X 3mL	X 3mL	X 3mL	X 3mL	X 3mL	X 3mL

1 – Screening laboratory can be performed up to 28 days prior to the scheduled clinic visit and includes: CBC with differential, AST, ALT, and HIV antibody. The screening laboratory results must be reviewed prior to the scheduled blood draw for the respective visit.

2 – CBC w/differential is to be performed on the scheduled clinic visit, with the blood draw for PBMC. It is intended for the back-calculation of the number and concentration of lymphocyte subsets.

3 – Not all volunteers will follow the same study schedule. The schedule will vary according to laboratory needs. No more than 550 mL will be drawn within a 56 day period.

Appendix C. Clinical Laboratory values, for study eligibility

Parameter	Values Leading to Study Exclusion	
	Low Outlier (or Change from Reference)*	**High Outlier (or Change from Reference)***
ALT (SGPT)	N/A	> 1.5 x ULN
AST (SGOT)	N/A	> 1.5 x ULN
WBC†	< 0.81 x LLN	> 1.09 x ULN
Hemoglobin†	M: < 0.92 x LLN F: < 0.91 x LLN	M: > 1.18 x ULN F: > 1.18 x ULN
Platelet (x 10^3) †	< 0.8 x LLN	> 1.2 x ULN
HIV	Positive	
Blood Type§	Not evaluated for eligibility (this test does not need to be repeated)	
For blood unit donations, the following lab values are exclusionary:‡		
WBC	<3.5 x10^3 /mm^3	>11 x10^3 /mm^3
Hemoglobin	<12.5 g/dL	>18 g/dL
Platelet Count	<150 x10^3 /mm^3	>500 x10^3 /mm^3

ULN = Upper limit of normal range

LLN = lower limit of normal range

N/A = not applicable

* Values that lead to exclusion are defined as either an absolute value based on the reference range provided by Garcia (the usual clinical laboratory used by CVD) or the deviation from the reference value of a particular clinical laboratory by the order of magnitude specified. "N/A" means that any lower value is acceptable for inclusion

† Note that different WBC, Hemoglobin, and Platelet Count criteria are defined, for those being evaluated for blood unit (large volume) blood donations

§ Blood Type is performed only once, at initial screening only

‡ to be performed by the Cell Component Therapy Facility at University of Maryland Greenebaum Cancer Center, as part of their standard operating procedures

Appendix D. Instructions for Vaxchora

Vaxchora
(Cholera Vaccine, Live, Oral)
Never underestimate cholera

Preparation, Reconstitution, and Administration

Prepare and administer Vaxchora in a health care setting equipped to dispose of medical waste.

To administer, follow the numbers.

1 dose
Vaxchora is administered as a single oral dose.

15 minutes
Once the carton containing the Vaxchora vaccine packet and the buffer packet has been removed from the freezer, the vaccine should be reconstituted within 15 minutes.

10 days
Vaxchora should be administered at least 10 days prior to potential exposure to cholera.

60 minutes
Patients should not eat or drink for 60 minutes before and 60 minutes after administration of Vaxchora.

15 minutes
Vaxchora must be consumed within 15 minutes of reconstitution.

Vaxchora should be reconstituted as follows:

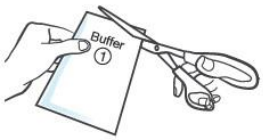
1

Reconstitution should be completed within 15 minutes of removing the carton from the freezer. Locate the 2 packets: the buffer component (Packet 1) and the active component (Packet 2).

2

Pour 100 mL of cold or room temperature (41°F-72°F; 5°C-22°C) purified bottled water into a clean, disposable cup. Do not use tap water, non-purified bottled water, other beverages, or other liquids.

3



Use scissors to cut the top off the buffer component packet.

4

Empty buffer component packet contents into cup. Effervescence will occur.

5

Using a disposable stirrer, stir until the buffer component completely dissolves.

6

Use scissors to cut the top off the active component packet.

7

Empty the active component packet contents (lyophilized *V. cholerae* CVD 103-HgR) into the cup containing the buffer solution.

8

Stir for at least 30 seconds and until active component disperses to form a slightly cloudy suspension that may contain some white particulates. The active component may not dissolve completely.

9

Vaxchora must be consumed within 15 minutes of reconstitution. The recipient should drink the full contents of the cup at once. Some residue may remain in the cup and should be discarded with the cup.

DISPOSE of the cup, packets, and stirrer according to standard procedures for medical waste.

Inactivate any spilled vaccine and clean any non-disposable equipment used in the preparation of Vaxchora with 70% isopropyl alcohol or 10% bleach solution.

NOTE: If the packets are reconstituted in the improper order, the vaccine must be discarded.

Storage

Vaxchora must be stored in a freezer between **-13°F and 5°F**. Protect from light and moisture. Packets do not require thawing prior to reconstitution.