CLINICAL STUDY PROTOCOL

PROTOCOL TITLE:	A Phase 4 Study to Assess the Safety and Immunogenicity of VAXCHORA (Cholera Vaccine, Live, Oral) in Children 2 to <18 Years of Age
PROTOCOL NUMBER:	PXVX-VC-200-006 (NCT03220737)
INVESTIGATIONAL PRODUCT:	VAXCHORA® (Cholera Vaccine, Live, Oral) IND 15010
SPONSOR:	PaxVax, Inc. [redacted] Redwood City, [redacted] [redacted]
SPONSOR MEDICAL MONITOR:	[redacted]
VERSION:	3.0
DATE:	15Nov2017

CONFIDENTIAL

IMPORTANT: This document contains proprietary and confidential information belonging to PaxVax, Inc. Acceptance of this document constitutes agreement by the recipient that no previously unpublished information contained herein will be published or disclosed without the prior written approval of PaxVax, Inc., except disclosure to study personnel and appropriate Institutional Review Boards (IRBs) and Institutional Biosafety Committees (IBCs), under the condition that such personnel have agreed to keep this information confidential. The foregoing shall not apply to disclosure required by governmental regulations or laws; however, PaxVax, Inc. shall be promptly notified of any such disclosure.

PROTOCOL SIGNATURE SHEET

A Phase 4 Study to Assess the Safety and Immunogenicity of VAXCHORA (Cholera Vaccine, Live, Oral) in Children 2 to <18 Years of Age

The information contained in this document and all information provided to you related to VAXCHORA™ (Cholera Vaccine, Live, Oral) are the confidential and proprietary information of PaxVax, Inc. (Sponsor) and except as may be required by federal, state or local laws or regulations, may not be disclosed to others without prior written permission of Sponsor. The Principal Investigator may, however, disclose such information to supervised individuals working on the Study, including individuals with any Institutional Review Boards (IRBs) or Institutional Biosafety Committees (IBCs), provided such individuals agree to be bound to maintain the confidentiality of such Study Vaccine information.

I agree to abide by the statement of confidentiality.

I agree to conduct the study according to this protocol. Any changes in procedure will only be made if necessary to protect the safety, rights, or welfare of subjects.

I agree to comply with the current International Conference on Harmonization Tripartite Guideline on Good Clinical Practice in addition to the appropriate FDA Code of Federal Regulations (CFRs) and state and local regulations.

I agree to conduct the study in person or to supervise the study.

I agree to ensure that all that assist me in the conduct of the study have access to the study protocol and any amendments and are aware of their responsibilities in meeting these obligations.

Principal Investigator
(Print Name)

Title

Signature **Date**

PROTOCOL SYNOPSIS

PROTOCOL TITLE	A Phase 4 Study to Assess the Safety and Immunogenicity of VAXCHORA™ (Cholera Vaccine, Live, Oral) in Children 2 to <18 Years of Age
SITES	Up to 30 sites in the US
INTRODUCTION	VAXCHORA (Cholera Vaccine, Live, Oral) is a vaccine indicated for active immunization against disease caused by *Vibrio cholerae* serogroup O1. VAXCHORA is approved for use in adults 18 through 64 years of age travelling to cholera-affected areas. The primary goals of this Phase 4 study are to evaluate the safety and immunogenicity of VAXCHORA in children ages 2 years to <18 years of age in developed countries.
BACKGROUND AND RATIONALE	Cholera is an acute enteric infection caused by the bacterium *V. cholerae* and transmitted by the ingestion of water or food containing the organism. Cholera is characterized in its most severe form (cholera gravis) by a sudden onset of acute watery diarrhea that can lead to severe dehydration and death. The *V. cholerae,* strain CVD 103-HgR was developed as a live, oral vaccine for the prevention of cholera in adults, and licensed in several countries ex-US under the trade names Orochol and Mutachol. Clinical trial experience with CVD 103-HgR includes administration to more than 27,000 adults and children as young as 3 months old, and over 500,000 commercial doses of CVD 103-HgR vaccine were sold with an indication in travelers two years or older. CVD 103-HgR was redeveloped by PaxVax and is now licensed under the trade name Vaxchora® (BLA STN 125597/0) for use in adults 18 through 64 years of age who are travelling to cholera-affected areas. Safety data from approximately 3,000 adult subjects in the VAXCHORA clinical development program show that there is very little difference in the frequency of reactogenicity and adverse events for recipients of the live-attenuated vaccine and recipients of placebo. Studies in children in both developed and developing countries using a single dose of the CVD 103-HgR vaccine strain showed that the vaccine was well tolerated. However, there are no published studies of serological response rates in children in developed countries.
ACTIVE VACCINE	VAXCHORA (Cholera Vaccine, Live, Oral) is a live, attenuated bacterial vaccine suspension for oral administration containing the *V. cholerae* strain CVD 103-HgR. CVD 103-HgR was constructed

	from the serogroup O1 classical Inaba strain 569B by deleting the catalytic domain sequence of both copies of the *ctxA* gene, which prevents the synthesis of active cholera toxin (CT). This attenuated strain remains able to synthesize the immunogenic non-toxic B subunit of CT (encoded by the *ctxB* gene). In addition, a marker was inserted into the hemolysin gene locus (*hlyA*) to enable differentiation of the vaccine strain from wild type *V. cholerae* O1. This nontoxic B subunit is immunogenic and capable of generating neutralizing antibodies against the toxic activity of the oligomeric cholera enterotoxin. Antibacterial serum vibriocidal antibodies (a surrogate of protection) that are directed mainly against the lipopolysaccharide O antigen (but also against surface protein antigens) are also induced by the live vaccine. VAXCHORA is provided as two single-use packets, one containing vaccine as lyophilized powder and one containing bicarbonate buffer. The contents of both packets are reconstituted in purified bottled water (Section 4.1). The resultant suspension is administered orally at a dose of approx. 1 x 10^9 colony forming units (CFU).
CONTROL VACCINE	The placebo control for this study is normal (0.9%) saline (Section 4.2).
MODE OF ADMINISTRATION	Subjects will take no food or water for 60 minutes before and after vaccination. VAXCHORA will be reconstituted in purified bottled water with buffer (Section 4.1). VAXCHORA and placebo will be administered orally.
OBJECTIVES	1) Demonstrate that the seroconversion rate at Day 11 in pediatric subjects is non-inferior to the seroconversion rate at Day 11 in adults between the ages of 18 and 45 years. 2) Demonstrate that the seroconversion rate in pediatric subjects is greater than or equal to 70 % with 98.3% confidence. The two primary objectives will be evaluated independently of one another in each of the 3 age groups of children according to a parallel sequential design with unified control of the total Type I error across all 6 tests. See the Alpha-Spending Strategy section for a description of the testing dependencies and the partitioning of the study wise Type I error.
STUDY POPULATION	Up to 595 healthy children ages 2 years to <18 years of age will be enrolled into three age Cohorts as follows: • Cohort 1: 12 to <18 years (n=150:active treatment group, n=25:placebo crossover group for 175 total subjects) • Cohort 2: 6 to <12 years (n=150:active treatment group, n=25:placebo crossover group for 175 total subjects) • Cohort 3: 2 to < 6 years (n=210:active treatment group, n=35:placebo crossover group for 245 total subjects)

	Cohorts may be enrolled concurrently. **Inclusion Criteria:** 1. Male or female 2. Between 2 and <18 years of age on Day 1. 3. In general good health. 4. Able and willing to provide informed assent for study participation. 5. Primary caregiver is able and willing to provide informed consent for study participation. 6. (for females of childbearing potential) Using an acceptable method of contraception through Day29. **Exclusion Criteria:** 1. Current acute gastrointestinal illness or loose stools within 3 days of Day 1 visit. 2. Current acute febrile illness. 3. History of cholera infection. 4. History of cholera vaccination. 5. History of severe allergic reaction (e.g. anaphylaxis) to any ingredient of VAXCHORA. 6. Congenital or acquired immunodeficiency. 7. Pregnancy (for females of childbearing potential) 8. Any other condition that, in the opinion of the Investigator, creates an unacceptable risk to the subject. 9. Any other condition that, in the opinion of the Investigator, will interfere with the conduct of the study or the validity of the data. 10. Duration of >2 weeks of abnormal stool pattern, defined as <3 stools per week or >2 stools per day in the past 6 months. 11. Regular use of laxatives in the past 6 months. 12. History of enterotoxigenic *E. coli* infection. 13. Travel to cholera-endemic area in the previous 5 years. 14. Nursing/breastfeeding. 15. Received or plans to receive the following from 14 days prior to the study vaccination through 11 days after vaccination: Any other licensed vaccines Antibiotics or chloroquine 16. Received or plans to receive any other investigational agent throughout the main study (Day 181).
STUDY DESIGN	This is a randomized, placebo-controlled, double-blind, single-crossover study with three age cohorts and two treatment groups within each cohort. Cohorts will be enrolled concurrently. The Safety Monitoring Committee (SMC) will conduct regular periodic reviews of all

	available interim, blinded safety data from simultaneous enrollment of all age cohorts. The main study consists of a screening period of 30 days, Day 1 vaccination, an observation period from Day 1 to Day 29, and a follow-up period through Day 181. Placebo crossover has an optional active vaccination at Day 181 after unblinding, with a 6 month post vaccination follow-up period (to Day 365). Cohort 1 has an optional sub-study consisting of a long-term follow-up period through Day 730. In each cohort, subjects will be randomized in a 6:1 ratio to the Active treatment group or the Placebo crossover group. The Active treatment group will receive VAXCHORA on Day 1 and the Placebo crossover group will receive placebo on Day 1. All subjects will have study visits at Days 1, 11, 29 and 181. Randomized treatment assignments will be unblinded at Day 181. Subjects in Cohort 1 will have in-clinic visits on Day 91. Subjects in Cohorts 2 and 3 will have follow-up calls on Day 91. Subjects in the Placebo crossover group will receive VAXCHORA after unblinding at Day 181 if they so choose and will then have study visits on Days 191 and 209 and phone calls at Days 271 and 365. After completion of the main study on Day 181, subjects in the active treatment group of Cohort 1 may participate (by separate informed assent and consent) in a long-term follow-up sub-study with study visits at Days 365, 547, and 730. The total duration of study participation is therefore up to 365 days (52 weeks) for the main study and up to 760 days (109 weeks) for Cohort 1 subjects who participate in both the main study and the sub-study. Study treatments are shown in Table 3. The Study Design is shown in Figure 1. Primary Comparator Group for All Age Cohorts: Healthy adult subjects, age 18-45 years who received a single dose of VAXCHORA while participating in the PXVX-VC-200-004 lot consistency trial.
STUDY PROCEDURES	**Safety:** Subjects will be monitored for acute reactions for 30 minutes after vaccination. Adverse events of abdominal pain, headache, lack of appetite, tiredness, diarrhea, nausea, vomiting and fever will be solicited daily through Day 8 (and from Day 181 through Day 188 for Placebo crossover subjects) using a memory aid (diary card). Unsolicited Adverse Events (AEs) will be collected through Day 29, and Day 181 through Day 209 (for Placebo crossover subjects). Serious Adverse Events (SAEs) will be collected for the duration of

	study participation. In Cohorts 2 and 3, events occurring after Day 29 will be assessed initially by telephone contact. **Immunogenicity:** Immunogenicity will be assessed by measurement of Serum Vibriocidal Antibodies (SVA) in all subjects at Days 1, 11, and 29. Subjects in Cohort 1 will have further measurements of SVA at Days 91 and 181. In the sub-study, subjects will have SVA measurements at Days 365, 547, and 730. Study procedures are shown in the Schedule of Events (Appendix A)
ENDPOINTS	**1) Cohort 1: 12 to <18 years** **Primary Endpoint:** • The proportion of subjects achieving seroconversion of SVA against the classical Inaba biotype of *V. cholerae* at Day 11 following one dose of VAXCHORA, defined as a 4-fold or greater rise over baseline Day 1 SVA titer. This proportion of seroconverters is called the seroconversion rate. **Secondary Endpoints:** • Seroconversion of SVA against the classical Inaba biotype of *V. cholerae* at Days 29, 91 and 181 for all subjects. • Seroconversion of SVA against the classical Inaba biotype of *V. cholerae* at Days 365, 547 and 730 for vaccinees participating in the sub-study. **Exploratory Endpoint: Associated memory B cell endpoints:** • Anti–O1 lipopolysaccharide (LPS) memory B cell concentration at Days 1, 91, 181 for the subjects in the active treatment group and the placebo crossover group, and Days 365, 547, 730 for the subjects in the active treatment group who participate in the sub-study. **2) Cohort 2: 6 to <12 years** **Primary Endpoint:** • The proportion of subjects achieving seroconversion of SVA against the classical Inaba biotype of *V. cholerae* at Day 11 following one dose of VAXCHORA, defined as a 4-fold or greater rise over baseline Day 1 SVA titer. **Secondary Endpoint:** • Seroconversion of SVA against the classical Inaba biotype of *V. cholerae* at Day 29 following one dose of VAXCHORA. **3) Cohort 3: 2 to <6 years**

	Primary Endpoint: • The proportion of subjects achieving seroconversion of SVA against the classical Inaba biotype of *V. cholerae* at Day 11 following one dose of VAXCHORA, defined as a 4-fold or greater rise over baseline Day 1 SVA titer. **Secondary Endpoint:** • Seroconversion of SVA against the classical Inaba biotype of *V. cholerae* at Day 29 following one dose of VAXCHORA. **Safety:** Evaluate the safety and tolerability of VAXCHORA using the following endpoints: • Solicited adverse events through Day 8: abdominal pain, headache, lack of appetite, tiredness, diarrhea, nausea, vomiting and fever, by age cohort and overall • Unsolicited adverse events through Day 29, by age cohort and overall • Serious adverse events through Day 181, by age cohort and overall **Acceptability:** Evaluate the acceptability of VAXCHORA using the following endpoint. • The percent of subjects in each age cohort able to complete the dosing according to protocol **Palatability:** Evaluate the palatability of VAXCHORA using the following endpoints: • Palatability of vaccine assessed by the subject using a 5-point Hedonic scale in Cohorts 1 and 2. • Palatability of vaccine assessed by the caregiver using a 5-point Hedonic scale in Cohort 3.
STATISTICAL METHODS	Solicited AEs will be summarized by the percent of subjects by treatment group who experience each solicited adverse event at least once. A second table will present a similar summary constrained to solicited AEs of moderate severity or higher. An additional analysis will summarize the number of days that subjects experience solicited AEs. Unsolicited AEs recorded through Day 29 will be summarized by treatment group, system organ class, preferred term and severity. A list of any SAEs recorded through Day 181 or later will be presented separately. Seroconversion will be defined as a 4-fold or greater rise in SVA over Day 1 (baseline). Each rate will be accompanied by a 95% confidence interval (CI) calculated using the Wilson method (Agresti 1998). Cumulative seroconversion through a particular

	visit is defined as the occurrence of seroconversion at or prior to that visit. SVA levels will be summarized by the geometric mean titer (GMT) of antibodies against the classical Inaba biotype of *V. cholerae* at all time points for which antibody assessments are available. Each GMT will be accompanied by a 95% confidence interval calculated by first constructing a confidence interval on log-transformed data and then back-transforming the endpoints of the confidence interval to the original data space. The relative increase in SVA at a given post-baseline timepoint will be summarized using the geometric mean of the fold-increase in titer (GMFI) between baseline and the timepoint. A 95% confidence interval for each GMFI will be constructed using the same log-transformation method as that used to build confidence intervals for GMTs. **Primary Analyses** 1) The seroconversion rate between Day 1 and Day 11 for vaccines in each of the 3 age cohorts will be compared to the seroconversion rate for vaccines between the ages of 18 and 45 who participated in the lot consistency study PXVX-VC-200-004 by calculating the difference between the two rates and computing a 96.7% confidence interval for this difference. 2) For each of the 3 age cohorts, the proportion of vaccinated subjects who experience a 4-fold or greater increase in serum vibriocidal titer between Day 1 and Day 11 will be calculated, and a 98.3% confidence interval on this proportion of seroconverters will be computed. **Acceptance Criteria for Primary Analyses** 1) The lower bound of the two-sided 96.7% confidence interval on the difference in seroconversion rates between children and adults must be greater than -10 percentage points. 2) The lower bound of the two-sided 98.3% confidence interval on the proportion of vaccinees who seroconvert between Day 1 and Day 11 must equal or exceed 70%. **Alpha-Spending Strategy** In order to ensure that the total Type I error for the study is capped at alpha = 0.05, 2/3 of the alpha will be allotted to the primary objective of establishing non-inferiority relative to adults, and 1/3 of the alpha will be allotted to the primary objective of demonstrating that the seroconversion rate equals or exceeds 70%. The two primary objectives will be evaluated independently, and within each primary objective, testing in the different age cohorts will proceed sequentially beginning with

	the data for the 12 to <18 age cohort, as follows: 1) Non-inferiority: the analysis of non-inferiority between vaccinees in the 12-18 age cohort and adults between the ages of 18 and 45 will be conducted first. Formal analysis of non-inferiority in the 6 to <12 age cohort will be performed only if the lower bound of the two-sided 96.7% confidence interval on the difference between the seroconversion rates for children in the 12 to <18 age cohort and adults equals or exceeds the pre-specified acceptance criterion. Formal evaluation of non-inferiority in the 2 to <6 age cohort will occur only if the acceptance criterion is met for both the 12 to <18 and 6 to <12 age cohorts. This strategy maintains the overall Type I error for the primary objective of non-inferiority at alpha = 0.033. 2) Seroconversion Rate: the analysis of the seroconversion rate among vaccinees in the 12 to <18 age cohort will be conducted first. Formal analysis of the acceptability of the seroconversion rate in the 6 to <12 age cohort will be performed only if the lower bound of the two-sided 98.3% confidence interval on the seroconversion rate in the 12 to <18 age cohort meets the pre-specified acceptance criterion. Formal evaluation of the acceptability of the seroconversion rate in the 2 to <6 age cohort will occur only if the acceptance criterion is met for both the 12 to <18 and 6 to <12 age cohorts. This strategy maintains the overall Type I error for the primary objective concerning the magnitude of the seroconversion rate at alpha =0.017. **Secondary Analyses** For vaccinees in the age cohort 12 to <18, the point estimate of the seroconversion rate for vaccinees as well as the cumulative seroconversion rate will be computed at the time points Day 29, Day 91 and Day 181, and a 95% confidence interval will be constructed for each proportion. Pointwise seroconversion rates, cumulative seroconversion rates, and confidence intervals for placebo recipients in the age cohort 12 to <18 will be computed at Day 11, Day 29, Day 91 and Day 181. For vaccinees in the age cohorts 2 to <6 and 6 to <12, pointwise and cumulative seroconversion rates and corresponding confidence intervals will be calculated for Day 29. Pointwise seroconversion rates, cumulative seroconversion rates, and confidence intervals for placebo recipients in the age cohorts 2 to <6 and 6 to <12 will be computed at Day 11 and Day 29. For vaccinees and placebo recipients in the age cohort 12 to <18, the GMT and 95% confidence interval will be computed for Day 1, Day 11, Day 29, Day 91 and Day 181, and the GMFI in each

	treatment group will be calculated for Day 11, Day 29, Day 91 and Day 181. For vaccinees and placebo recipients in the age cohorts 2 to <6 and 6 to <12, GMTs and 95% confidence intervals will be calculated for Day 1, Day 11 and Day 29, and GMFIs and corresponding confidence intervals will be calculated for Day 11 and Day 29. For participants in the sub-study, the GMT and 95% confidence interval and GMFI with 95% confidence interval will be calculated for Days 365, 547 and 730. **Memory B Cell Concentration Analyses** In the main study, anti-O1 LPS memory B cells will be assessed at Days 1, 91 and 181 for a subset of subjects in Cohort 1 and at Days 365, 547, and 730 for subjects in the active treatment group in Cohort 1 who are participating in the sub-study. Point estimates of median percentage as well as median fold rise from Day 1 to Day 91, Day 181, 365, 547, 730 will be presented. **Sample Size Rationale** Assuming that the true seroconversion rate among 12 to <18-year-olds is 92.4% or higher, the sample size of 143 evaluable vaccinees for that age cohort affords 93.3% power to demonstrate that the seroconversion rate within the group is non-inferior to the 94% rate observed in the 2687 adult subjects assessed in the PaxVax lot-consistency trial, PXVX-VC-200-004. The sample size to be enrolled allows for up to 5% of subjects not evaluable for the primary immunogenicity analyses in the two older cohorts (ages 6 to < 18). Based on the inevaluable rate observed to date, it is estimated that approximately 30% of subjects in the youngest cohort (ages 2 to < 6) will be inevaluable, mostly due to lack of consumption of the full dose. Making similar assumptions about the true seroconversion rates in the other two pediatric age cohorts, and assuming that the rates in the three age cohorts are independent of one another, the overall power for demonstrating the non-inferiority of all three age cohorts is $(93.3\%)^3 = 81\%$. Under the same assumptions as above – in particular, assuming the true seroconversion rate within an age cohort is at least 92.4% – 143 evaluable vaccinees provide greater than 99.9% power to establish that the lower bound on the cohort-specific rate is at least 70%. Power is still greater than 99.9% when requiring the lower bound on all three cohorts to be 70% or greater. The overall power of meeting both primary objectives in all three age cohorts can be approximated by multiplying the power of meeting the non-inferiority objective by the power of meeting the 70% lower bound objective: 81% x 99.9% ≈ 81%. Given a total of 510 subjects receive VAXCHORA, there is a 99% chance that an uncommon AE - one expected to occur in only 1% of vaccinees - will be observed at least once during the trial.

	Interim Analysis After the last subjects enrolled in Cohorts 1 and 2 have completed their Day 181 visit, a preliminary immunogenicity and safety analysis may be performed and report written to facilitate a regulatory submission.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AE	Adverse Event
BLA	Biologics License Application
CFU	Colony Forming Unit
CI	Confidence Interval
CRF	Case Report Form
CT	Cholera Toxin
CVD	Center for Vaccine Development
DMP	Data Management Plan
eCRF	electronic Case Report Form
EDC	Electronic Data Capture
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GMFI	Geometric Mean Fold Increase
GMT	Geometric Mean Titer
IBC	Institutional Biosafety Committee
ICF	Informed Consent Form
ICH	International Conference on Harmonization
IND	Investigational New Drug Application
IPD	Important Protocol Deviations
IRB	Institutional Review Board
LPS	lipopolysaccharide
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SMM	Sponsor Medical Monitor
SMP	Safety Management Plan
SVA	Serum Vibriocidal Antibody
TMF	Trial Master File
US	United States
VAXCHORA	VAXCHORA (Cholera Vaccine, Live, Oral)
V. cholerae	*Vibrio cholerae*
WHO	World Health Organization

1 INTRODUCTION

1.1 Background

Cholera is an acute enteric infection caused by the bacterium *V. cholerae* O1 or O139 and is transmitted by the ingestion of water or food containing the organism. The illness principally occurs in countries with insufficient access to safe water and proper sanitation, with even more dramatic impact in areas where basic environmental infrastructures are disrupted or have been destroyed.

Cholera is characterized in its most severe form (cholera gravis) by a sudden onset of acute electrolyte-rich watery diarrhea that can lead to severe dehydration and death. The extremely short incubation period—approximately 12 hours to 5 days—enhances the potentially explosive pattern of outbreaks, as the number of cases can rise very quickly.

Cholera remains an important public health concern with focus on developing countries. In 2014, a total of 190,549 cholera cases with 2,231 deaths were reported to WHO by 42 countries resulting in an overall case fatality rate (CFR) of 1.17%. Compared to 2013, this represents a 47% increase (WHO 2015). Deaths due to cholera were reported by 24 counties; 1882 deaths occurred in Africa, 42 in Asia, and 307 in Hispaniola. Imported cases were reported from 11 countries including the USA and Canada in 2014. It is estimated that 1.3-4.0 million cholera cases, with 21,000-143,000 deaths, occur each year worldwide (Ali 2015). Under-reporting is often due to fear of negative impact on travel and trade in the affected area and limitations in surveillance systems, inconsistencies in case definitions and lack of laboratory diagnostic capacities. The persistence of cholera in many countries in Asia and Africa, the appearance of particularly severe clinical disease due to El Tor strains expressing classical biotype cholera toxin, and the increasing prevalence of antimicrobial resistance make the control of cholera a high priority on the public health agenda. Two WHO prequalified Oral Cholera Vaccines (OCVs) are currently available on the international market (Dukoral® [Crucell; Leiden, The Netherlands] and Shanchol [Shantha Biotechnics; Hyderabad, India])) for individuals aged ≥ 1 year and older.

The *V. cholerae,* strain CVD 103-HgR was developed as a live, oral vaccine for the prevention of cholera in adults, and licensed in several countries ex-US under the trade names Orochol and Mutachol. Clinical trial experience with CVD 103-HgR includes administration to more than 27,000 adults and children as young as 3 months old, and over 500,000 commercial doses of CVD 103-HgR vaccine were sold with an indication in travelers two years or older.

The same strain was redeveloped by PaxVax and is now licensed for use under the trade name VAXCHORA® (BLA STN 125597/0). In June 2016 VAXCHORA was approved by the FDA for use in adults 18 through 64 years of age travelling to cholera-affected areas.

Studies in children in both developed and developing countries using a single dose of the CVD 103-HgR vaccine strain showed the vaccine was well tolerated and suggested that the immune response (measured by vibriocidal seroconversion rate and GMT) to a single dose of vaccine was lower than the responses in adults.

Table 1: Orochol Pediatric Trials

Country	Age	Dose (CFU)	N	Reference
Indonesia	5 to 9 y	Up to 1 x 10^{10}	412	Suharyono 1992
Indonesia	2 to 5 y	5 x 10^9	~150	Simanjuntak 1993
Chile	5 to 9 y	5 x 10^9	178	Lagos 1995
Chile	2 to 4 y	5 x 10^9	100	Lagos 1996
Chile	3 to 17 mo	5 x 10^9	312	Lagos 1999a
Austria	6 mo to 12 y	5 x 10^8	29	Wiedermann 1998
Indonesia	2 to 18 y	5 x 10^9	>15,000	Richie 2000
Ecuador	6 to 13 y	5 x 10^8	139	Cooper 2000
Gabon	4 to 16 y	5 x 10^9	275	Faucher 2002

In 349 healthy 5-9 year old Chilean children given 5 x 10^9 CFU of CVD 103-HgR (n = 178) or placebo (n = 171), the vaccine was well tolerated and none of the adverse events occurred at a significantly greater rate than in the placebo group (CVD 31002, Lagos 1995). In an ensuing phase, the age of the study subjects was lowered to 2-4 years (CVD 31003, Lagos 1996). In a double-blind randomized study, 100 children received a single dose of CVD 103-HgR containing 5x 10^9 CFU and 100 paired siblings a dose of placebo. Again, there was no significant difference between the incidence of diarrhea or any other adverse events in the vaccine and placebo groups.

Although Orochol was used in children in Europe, Canada, and Australia, there are no published studies of the serological response rates in children in developed countries. Immune responses to oral vaccines may be very different in infants and children in endemic countries due to e.g. natural exposure to infections, age-related differences in immune function, breast feeding and nutritional status (Czerkinsky 2015, Sack 2008). Therefore, this study is designed to determine the vibriocidal antibody response rates in children in developed countries following a single dose of VAXCHORA.

1.1.1 Name and Description of Active Vaccine

VAXCHORA®™ (Cholera Vaccine, Live, Oral) is a live, attenuated bacterial vaccine suspension for oral administration indicated for active immunization against disease caused by *Vibrio cholerae* serogroup O1 in adults 18 through 64 years of age traveling to cholera-affected areas. It contains the *V. cholerae* strain CVD 103-HgR. CVD 103-HgR was constructed from the serogroup O1 classical Inaba strain 569B by deleting the catalytic domain sequence of both copies of the *ctxA* gene, which prevents the synthesis of active cholera toxin (CT). This attenuated strain remains able to synthesize the immunogenic non-toxic B subunit of CT (encoded by the *ctxB* gene). In addition, a marker was inserted into the hemolysin gene locus (*hlyA*) to enable differentiation of the vaccine strain from wild type *V. cholerae* O1.

Each dose of VAXCHORA is supplied as a foil packet of buffer and an accompanying foil packet of the active component (lyophilized *V. cholerae* CVD 103-HgR) which are reconstituted in purified bottled water to form a single dose according to the instructions in Section 4.1.

The vaccine strain is grown in fermentors under controlled conditions in medium containing casamino acids, yeast extract, mineral salts, and an anti-foaming agent. The bacteria are collected by filtration, diafiltered, and concentrated before addition of a stabilization solution containing ascorbic acid (an antioxidant), Hy-Case SF (hydrolyzed casein [a protein derived from cow's milk], a cryoprotectant), sodium chloride (a stabilizer), and sucrose (a cryoprotectant).The stabilized bacteria are lyophilized, milled, and blended with dried lactose (a desiccant and bulking agent). The active component blend is filled into packets.

The buffer component is manufactured by blending together sodium bicarbonate (a gastric acid neutralizer), sodium carbonate (a buffer), ascorbic acid (a buffer and water chlorine neutralizer), and dried lactose (a manufacturing flow aid). The buffer component blend is filled into packets. One buffer component packet and one active component packet are packaged into individual single dose cartons for distribution.

After reconstitution, VAXCHORA contains 4 x 10^8 to 2 x 10^9 colony forming units (CFU) of live attenuated *V. cholerae* CVD 103-HgR. The resulting suspension should be slightly cloudy and may contain white particulates. The active and buffer ingredients are shown in Table 2 (VAXCHORA 2016).

Table 2: Vaccine Composition

Ingredient	Quantity/packet
Active Component Packet	
V. cholerae CVD 103-HgR	4 x 10^8 to 2 x 10^9 CFU
Sucrose	≤ 165.37 mg
Sodium chloride	≤ 17.11 mg
Hy-Case SF (hydrolyzed casein)	≤ 17.11 mg
Ascorbic acid	≤ 8.55 mg
Dried lactose	≤ 2.09 g
Buffer Component Packet	
Sodium bicarbonate	2.16–2.41 g
Sodium carbonate	0.24-0.49 g
Ascorbic acid	1.50–1.80 g
Dried lactose	0.18–0.22 g

CFU=colony forming units
mg= milligrams
g=grams

1.1.2 Summary of Findings from Nonclinical Studies

V. cholerae O1 is a strictly human pathogen, hence there is no valid animal model available to assess safety or predict the mucosal immune response to a live attenuated cholera vaccine (Richardson 1994). Cholera bacilli will not colonize or replicate in healthy adult animals, therefore animal studies would neither be expected to identify potential toxicity from their replication nor immune response to their replication. The only animal models of cholera disease are rabbits that

have been surgically modified using ligated ileal loops (Formal 1961) or the Removable Intestinal Tie-Adult Rabbit (RITARD) model (Pierce 1988, Dziejman 2005, Russell 1992, Morris 1990), which do not provide a relevant model for single or repeat dose, carcinogenicity or reproductive toxicology studies of this vaccine.

1.1.3 Summary of Findings from Clinical Studies

Safety

The safety of VAXCHORA was evaluated in four randomized, placebo-controlled, multicenter clinical trials. A total of 3235 adults 18 through 64 years of age received one dose of VAXCHORA and 562 received placebo [physiologic saline (N=551) or lactose (N=11)]. Overall, the mean age was 32.5 years; 53.8% of trial participants were female; 67.1% were White, 27.3% were Black or African American, 1.8% were Asian, 1.7% were multiracial, 1.3% were other, 0.6% were American Indian or Alaskan Native and 0.3% were Native Hawaiian or Pacific Islander. There were 9.3% Hispanic or Latino participants.

Adults 18 through 45 years of age received VAXCHORA in a multi-center, double-blind, randomized (8:1), placebo-controlled trial conducted in the United States and Australia. The safety analysis set included 2789 VAXCHORA recipients. Solicited adverse reactions were recorded daily for 7 days following vaccination. The most common adverse reactions (incidence > 3%) were tiredness (31%), headache (29%), abdominal pain (19%), nausea/vomiting (18%), lack of appetite (17%) and diarrhea (4%).

In a pooled analysis of the four clinical studies, 0.6% (20/3235) of VAXCHORA recipients and 0.5% (3/562) of placebo recipients reported a serious adverse event within 6 months post-vaccination. None of these events were considered to be related to vaccination. Refer to the VAXCHORA package insert for a complete summary of findings from clinical studies (VAXCHORA 2016).

Protective Efficacy

In a randomized, double-blind, saline placebo-controlled *V. cholerae* challenge study conducted in the US, subjects 18 through 45 years of age (N=197) with no prior history of cholera infection or travel to a cholera-endemic area in the previous 5 years were randomized according to a 1:1 ratio to receive one dose of VAXCHORA or placebo. The challenges were split into 2 cohorts for 10 day and 3 month challenges. Subjects were admitted to an inpatient unit. The oral challenge consisted of 1 x 10^5 CFU live wild type *V. cholerae* El Tor Inaba N16961 in 30 mL $NaHCO_3$ buffer at 10 days or 3 months post-vaccination. The co-primary objectives were to demonstrate the efficacy of a single dose of VAXCHORA in the prevention of moderate to severe diarrhea following challenge at 10 days and 3 months post-vaccination. Moderate to severe diarrhea was defined as cumulative diarrheal purge ≥ 3 liters (L) within 10 days after challenge. Diarrheal stool was defined as ≥ 2 unformed stools (takes shape of container) collected during a 48 hour period weighing ≥ 200 grams (g) or a single unformed stool ≥ 300 g. VAXCHORA recipients challenged at 10 days post-vaccination and VAXCHORA recipients challenged at 3 months post-vaccination were compared with a pooled group of placebo (saline) recipients challenged at 10 days or 3 months post-vaccination.

Of the 95 VAXCHORA recipients, 68 were challenged; 35 were challenged at 10 days post-vaccination and 33 were challenged at 3 months post-vaccination. Of the 102 placebo recipients, 66 were challenged; 33 were challenged at 10 days post-vaccination and 33 at 3 months post-vaccination. Vaccine efficacy against the occurrence of moderate to severe diarrhea at 10 days post-vaccination was 90.3% [95% CI 62.7%, 100.0%] and at 3 months post-vaccination was 79.5% [95% CI 49.9%, 100.0%]

Immunogenicity

A vibriocidal antibody assay was used to measure serum levels of neutralizing antibodies against the vaccine strain. In the subset of subjects challenged in the cholera challenge study, 91% [95% CI 82%, 97%] of vaccinees seroconverted prior to challenge and 9% developed moderate to severe cholera following challenge, while 2% of placebo recipients seroconverted prior to challenge and 59% developed moderate to severe cholera following challenge. (Seroconversion was defined as a $\geq$ 4-fold rise in serum vibriocidal antibody from baseline to 10 days post-vaccination.) Based on the observed association between seroconversion and protection from *V. cholerae* disease, seroconversion rate at 10 days post-vaccination was used to evaluate response to vaccination in other age cohorts.

In a randomized, double-blind, saline placebo-controlled safety and immunogenicity study conducted in the US and Australia, a total of 3146 subjects 18 through 45 years of age not previously exposed to cholera were randomized 8:1 to receive one dose of VAXCHORA or placebo. In this study, the rates of seroconversion were 93.5% [95% CI 92.5%, 94.4%] in vaccine recipients and 4% [95% CI 2%, 7%] in placebo recipients at 10 days post-vaccination.

In a randomized, double-blind, placebo-controlled safety and immunogenicity study conducted in the US, a total of 398 subjects 46 through 64 years of age with no prior history of cholera infection or travel to a cholera endemic area in the previous 5 years were randomized 3:1 to receive one dose of VAXCHORA or placebo.

Seroconversion rates at 10 days post-vaccination by classical Inaba vibriocidal antibody among 46 through 64 year old subjects were compared to those in 18 through 45 year old subjects. Adults 46 through 64 years were shown to have a non-inferior rate of seroconversion by classical Inaba vibriocidal antibody at 10 days post vaccination compared to adults 18 through 45 years of age. Refer to the VAXCHORA package insert for a complete summary of findings from clinical studies (VAXCHORA 2016).

1.2 Rationale for the Current Study

1.2.1 Rationale for Dosage and Route of Administration

VAXCHORA is an orally administered vaccine that is prepared and administered in a healthcare setting. Each dose of VAXCHORA is supplied as a foil packet of buffer and an accompanying foil packet of the active component (lyophilized *V. cholerae* CVD 103-HgR) which are reconstituted in purified bottled water to form a single dose. Each vaccine packet contains 4 x 10^8 to 2 x 10^9 CFU of live attenuated *V. cholerae*. Refer to Section 4.1 for reconstitution and administration instructions.

1.2.2 Rationale for Pediatric Study

Previous pediatric clinical trial experience with CVD 103-HgR involved the Orochol E® formulation that contained approximately 5 x 10^9 CFU/dose. A single dose of Orochol E elicited high rates of seroconversion in subjects with low or absent baseline titers of vibriocidal antibody, with a safety profile similar to that observed in adults (Orochol package insert 2000). In areas where cholera was endemic or where outbreaks had occurred, children who did not seroconvert already had elevated baseline vibriocidal titers. These pediatric studies with Orochol E were accepted by regulatory agencies in the licensure of CVD 103-HgR in the pediatric population 2 years of age and higher.

Compared with older children, younger children have similar rates of serum vibriocidal antibody seroconversion but lower GMTs, as demonstrated in Indonesia (Simanjuntak 1993, Lagos 1999a) and Chile (Lagos 1999a). Since these clinical trials were carried out in either developing countries or in sub-populations in transitional countries, many factors can contribute to the difference, including socioeconomic status, environmental enteropathy (measured as small bowel bacterial overgrowth, Lagos 1999b), geohelminths and early *H. pylori* gastritis (Levine 2010).

Since these factors would likely not be present in the US and other developed countries, it is possible that children from these countries may mount more substantial immune responses to VAXCHORA. This trial represents the first evaluation of the immunogenicity of any formulation of CVD 103-HgR in such children. This trial is designed to enable the independent evaluation of the immunogenicity of VAXCHORA in separate age cohorts: 12 to <18 years, 6 to <12 years, and 2 to <6 years.

2 OBJECTIVES AND ENDPOINTS

2.1 Immunogenicity Objectives and Endpoints

1) Cohort 1: 12 to <18 years

Primary Immunogenicity Objectives:

- Demonstrate that the seroconversion rate at Day 11 in pediatric subjects is non-inferior to the seroconversion rate at Day 11 in adults between the ages of 18 and 45 years.
- Demonstrate that the seroconversion rate in pediatric subjects is greater than or equal to 70% with 98.3% confidence.

Primary Endpoint:

- The proportion of subjects achieving seroconversion of serum vibriocidal antibody (SVA) against the classical Inaba biotype of *V. cholerae* at Day 11 following one dose of VAXCHORA, defined as a 4-fold or greater rise over baseline Day 1 SVA titer. This proportion of seroconverters is called the seroconversion rate.

Secondary Endpoints:

- Seroconversion of SVA against the classical Inaba biotype of *V. cholerae* at Days 29, 91 and 181 for all subjects.
- Seroconversion of SVA against the classical Inaba biotype of *V. cholerae* at Days 365, 547 and 730 for vaccinees participating in the sub-study.

Exploratory Objective:

- Explore memory B cell response to VAXCHORA vaccination at each time point.

Exploratory Endpoint:

- Associated memory B cell endpoints: Anti–O1 lipopolysaccharide (LPS) memory B cell concentration at Days 1, 91, 181 for the subjects in the active treatment group and the placebo crossover group and Days 365, 547, 730 for the subjects in the active treatment group who participate in the sub-study.

2) Cohort 2: 6 to <12 years

Primary Immunogenicity Objectives:

- Demonstrate that the seroconversion rate at Day 11 in pediatric subjects is non-inferior to the seroconversion rate at Day 11 in adults between the ages of 18 and 45 years.
- Demonstrate that the seroconversion rate in pediatric subjects is greater than or equal to 70% with 98.3% confidence.

Primary Endpoint:

- The proportion of subjects achieving seroconversion of serum vibriocidal antibody (SVA) against the classical Inaba biotype of *V. cholerae* at Day 11 following one dose of VAXCHORA, defined as a 4-fold or greater rise over baseline Day 1 SVA titer.

Secondary Endpoint:

- Seroconversion of serum vibriocidal antibody (SVA) against the classical Inaba biotype of *V. cholerae* at Day 29 following one dose of VAXCHORA.

3) Cohort 3: 2 to <6 years

Primary Immunogenicity Objectives

- Demonstrate that the seroconversion rate at Day 11 in pediatric subjects is non-inferior to the seroconversion rate at Day 11 in adults between the ages of 18 and 45 years.
- Demonstrate that the seroconversion rate in pediatric subjects is greater than or equal to 70% with 98.3% confidence.

Primary Endpoint:

- The proportion of subjects achieving seroconversion of serum vibriocidal antibody (SVA) against the classical Inaba biotype of *V. cholerae* at Day 11 following one dose of VAXCHORA, defined as a 4-fold or greater rise over baseline Day 1 SVA titer.

Secondary Endpoint:

- Seroconversion of serum vibriocidal antibody (SVA) against the classical Inaba biotype of *V. cholerae* at Day 29 following one dose of VAXCHORA.

2.2 Safety Objective and Endpoints

Objective:

- Evaluate the safety and clinical acceptability of VAXCHORA in children.

Endpoints:

Evaluate the safety and tolerability of VAXCHORA using the following endpoints:

- Solicited adverse events through Day 8: abdominal pain, headache, lack of appetite, tiredness, diarrhea, nausea, vomiting and fever, by age cohort and overall
- Unsolicited adverse events through Day 29, by age cohort and overall
- Serious adverse events through Day 181, by age cohort and overall.

2.3 Acceptability

Objective:

- Evaluate the acceptability of VAXCHORA using the following endpoint.

Endpoint:

- The percent of subjects in each age cohort able to complete dosing according to protocol.

2.4 Palatability

Objective:

- Evaluate the palatability of VAXCHORA using the following endpoints.

Endpoints:

- Palatability of vaccine assessed by the subject using a 5-point Hedonic scale in Cohorts 1 and 2.
- Palatability of vaccine assessed by the caregiver using a 5-point Hedonic scale in Cohort 3.

3 STUDY PLAN

3.1 Study Design

This is a randomized, placebo-controlled, double-blind, single-crossover study with three age cohorts and two treatment groups within each cohort enrolled concurrently (Figure 1).

Figure 1. PXVX-VC-200-006 Study Design

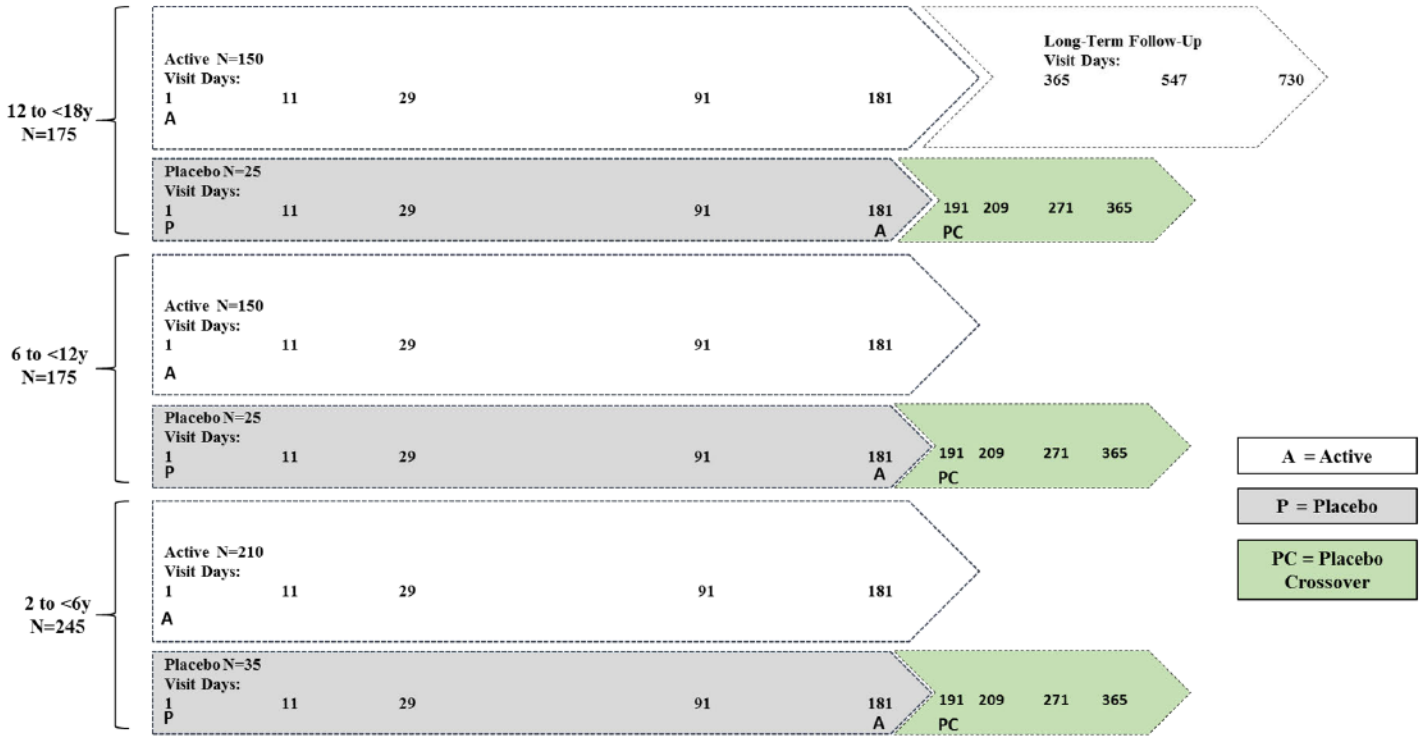

Table 3: PXVX-VC-200-006 Study Treatments by Cohort and Treatment Group

Cohort	Age (years)	Treatment Group	N	Day 1 Treatment (blinded)	Day 181 Treatment (Placebo crossover)
1	12 to <18	Active	150	VAXCHORA	None
		Placebo crossover	25	Placebo	VAXCHORA
2	6 to <12	Active	150	VAXCHORA	None
		Placebo crossover	25	Placebo	VAXCHORA
3	2 to <6	Active	210	VAXCHORA	None
		Placebo crossover	35	Placebo	VAXCHORA
		Total	**595**		

3.2 Number of Study Participants

Up to 595 healthy children ages 2 years to <18 years of age will be enrolled by age cohort as follows:

- Cohort 1: 12 to <18 years (n=150: active treatment group, n=25: placebo crossover group for 175 total subjects)
- Cohort 2: 6 to <12 years (n=150: active treatment group, n=25: placebo crossover group for 175 total subjects)
- Cohort 3: 2 to < 6 years (n=210: active treatment group, n=35: placebo crossover group for 245 total subjects)

Cohorts may be enrolled concurrently.

3.3 Estimated Study Duration

The main study consists of a screening period of 30 days, Day 1 vaccination, an observation period from Day 1 to Day 29, and a follow-up period through Day 181. Placebo crossover has an optional active vaccination at Day 181 after unblinding, with a 6 month of post vaccination follow-up period (to Day 365). Cohort 1 has an optional sub-study consisting of a long-term follow-up period through Day 730.

3.4 Study Population

3.4.1 Inclusion Criteria

Subjects must meet all of the following criteria in order to be enrolled:

1. Male or female.
2. Between 2 and <18 years of age on Day 1.
3. In general good health.
4. Able and willing to provide informed assent for study participation.
5. Primary caregiver is able and willing to provide informed consent for study participation.
6. (for females of childbearing potential) Using an acceptable method of contraception through Day 29 (see Section 5.9).

3.4.2 Exclusion Criteria

Subjects who meet any of the following criteria cannot be enrolled:

1. Current acute gastrointestinal illness or loose stools within 3 days of Day 1 visit.
2. Current acute febrile illness.
3. History of cholera infection.
4. History of cholera vaccination.
5. History of severe allergic reaction (e.g. anaphylaxis) to any ingredient of VAXCHORA.
6. Congenital or acquired immunodeficiency.
7. (for females of childbearing potential) Pregnant
8. Any other condition that, in the opinion of the Investigator, creates an unacceptable risk to the subject.
9. Any other condition that, in the opinion of the Investigator, will interfere with the conduct of the study or the validity of the data.

10. Duration of >2 weeks of abnormal stool pattern, defined as <3 stools per week or >2 stools per day in the past 6 months.
11. Regular use of laxatives in the past 6 months.
12. History of enterotoxigenic *E. coli* infection.
13. Travel to cholera-endemic area in the previous 5 years.
14. Nursing/breastfeeding.
15. Received or plans to receive the following from 14 days prior to the study vaccination through 11 days after vaccination:

    Any other licensed vaccines
    Antibiotics or chloroquine or any other investigational agents.
16. Received or plans to receive any other investigational agent throughout the main study (Day 181).

4 STUDY VACCINE

4.1 VAXCHORA

VAXCHORA (Cholera Vaccine, Live, Oral) is a live, attenuated bacterial vaccine suspension for oral administration indicated for active immunization against disease caused by *Vibrio cholerae* serogroup O1 in adults 18 through 64 years of age traveling to cholera-affected areas. It contains the *V. cholerae* strain CVD 103-HgR. CVD 103-HgR, constructed from the serogroup O1 classical Inaba strain 569B by deleting the catalytic domain sequence of both copies of the *ctxA* gene, which prevents the synthesis of active cholera toxin (CT). This attenuated strain remains able to synthesize the immunogenic non-toxic B subunit of CT (encoded by the *ctxB* gene). In addition, a marker was inserted into the hemolysin gene locus (*hlyA*) to enable differentiation of the vaccine strain from wild type *V. cholerae* O1.

Each dose of VAXCHORA is supplied as a foil packet of buffer and an accompanying foil packet of the active component (lyophilized *V. cholerae* CVD 103-HgR) which are reconstituted in purified bottled water to form a single dose. The resultant suspension is administered at a dose of approximately 4×10^8 to 2×10^9 colony forming units (CFU). Subjects will take nothing by mouth (food and water) for 60 minutes before and after vaccination.

Refer to the current version of the PXVX-VC-200-006 Pharmacy Manual for detailed preparation, reconstitution, administration and storage of the study vaccine.

For Subjects aged 6 years and older (Cohort 1 and 2):

Reconstitution should be completed within 15 minutes of removing the carton from the freezer. VAXCHORA will be prepared by suspending the contents of the buffer sachet into an opaque container containing 100 mL purified bottled water provided by PaxVax, and stirring the final suspension for at least 30 seconds. VAXCHORA must be consumed within 15 minutes of reconstitution. The subject should drink the full contents of the cup at once.

For Subjects younger than 6 years (Cohort 3):

Vaccine will be prepared by suspending the contents of the buffer sachet into an opaque container containing 100 mL of purified bottled water provided by PaxVax, then, discarding 50 mL of the

buffer suspension. This will halve the volume of buffer administered to the youngest children. The vaccine sachet is then emptied into the opaque container containing 50 mL of the buffer solution, and the mixture is stirred for at least 30 seconds. VAXCHORA must be consumed within 15 minutes of reconstitution. Each subject will imbibe the 50 mL of liquid to which she/he is allocated.

4.2 Placebo

Placebo will be 100 mL (for Cohort 1 and 2) and 50 mL (for Cohort 3) normal (0.9%) saline. The placebo will not be matched to the vaccine visually or by taste. Placebo (physiological saline) will be dispensed in an opaque cup. Subjects will be asked not to discuss the taste of the product with other study subjects.

4.3 Study Vaccine Accountability

PaxVax will supply study vaccine (VAXCHORA or placebo), purified bottled water and opaque cups to all study sites.

It is the responsibility of the Investigator to supervise accurate monitoring of the receipt, storage, dispensing, and accounting of all study material according to accepted medical and pharmaceutical practice. Sites must retain accurate, original site records of study material inventory as well as copies of all invoices of study material shipments and records of study material disposition.

Drug accountability forms will be used for tracking study material (e.g., date material was received, dispensed to individual subjects, amount used and unused on site, etc.).

Each site must keep all unused study material until the Study Monitor either arranges return to PaxVax or their designee or gives instruction for their disposal.

4.4 Study Vaccine Dispensing

The Unblinded Pharmacist (or designated study staff) will dispense study vaccine to the unblinded Dose Administrator according to each subject's treatment assignment. Subjects should be dosed by the unblinded Dose Administrator at Day 1. In some cases, the functions of the Unblinded Pharmacist and Dose Administrator may be performed by the same person. Each site must keep a Site-Specific Blinding Plan and responsible individuals must sign on the plan.

Unblinded Pharmacist (or designated staff) and unblinded Dose Administrator will not be involved in post-vaccination assessments and evaluation of VAXCHORA acceptability and palatability.

5 STUDY PROCEDURES

5.1 Informed Consent

Prior to any study-related activities, the subject's parent or legal guardian must sign and date an Institutional Review Board (IRB)-approved Informed Consent Form (ICF). Subjects within the older Cohort 1 (≥12 to <18 y) will provide written assent. Subjects in Cohort 2 may provide written assent per each IRB's recommendation. Refer to Section 10.3 for ICF guidelines.

5.2 Screening Procedure

All subjects will undergo screening procedures after at least one parent or legal guardian has given their written, informed consent for their child to participate in the study. Subjects in the oldest age cohort (≥12 to <18 y) will additionally provide their written assent to participate in the study.

The study centers will assign a unique, sequential subject number to each subject who provides written consent. Upon completion of all Screening Visit procedures (Section 6.1.1) and determination of eligibility, subjects will be scheduled to return for the Day 1 Visit. Screening Visit procedures must be completed no more than 30 days before the Day 1 Visit, but may be performed the same day as the Day 1 visit.

A screening log will be maintained at the clinical research site to record the reason(s) for exclusion from study for all subjects who sign the informed consent and undergo screening.

5.3 Re-Screening Procedure

There may be situations in which a subject is not able to be randomized within 30 days of their initially scheduled Day 1 Visit. In these instances, the subject may need to be re-screened. This will involve undergoing all screening procedures again. Subjects may be re-screened one time only.

5.4 Randomization Procedure

On Day 1 pre-vaccination, medical history and concomitant medications will be updated in the subject file and negative urine pregnancy test confirmed, if applicable, and blood collected for immune response assessments. The Investigator will confirm and document the eligibility of each subject immediately prior to randomization. Subjects will be considered enrolled once a randomization number has been assigned. Delegated site staff users will enroll each subject by assigning and entering a Subject ID # in the Electronic Data Collection (EDC) system. The EDC user will access the integrated randomization function within the EDC system to randomize the subject to one of the treatment arms. Subjects will be randomized at up to 30 clinical research sites. The assigned treatment will appear only on the randomization schedule that will be delivered to unblinded study personnel before they enroll subject(s) at their site. Blinded study personnel will print the randomization number for each subject from the EDC randomization form and take it to the unblinded personnel, who will match the randomization number to the randomization schedule and dispense the associated treatment.

The study will be conducted as a double-blind study through Day 181, where neither the sponsor, the statistical team, study volunteer subjects, nor clinical site personnel (except for the unblinded staff), will know subjects' treatment assignment. Once each subject has reached their Day 181 visit

they will be individually unblinded. Once all safety and immunogenicity data have been received from the Day 181 visit and the data cleaned, and signed by the PI, preventing further changes, the biostatistics group will carry out the primary analysis. Subjects, clinical site personnel, Investigators and the sponsor will remain blinded until each subject has completed their Day 181 visit. Each subject is unblinded individually at Day 181 and at that time their active/placebo status will be recorded in the EDC. Refer to the current version of PXVX-VC-200-006 Unblinding Plan.

5.5 Blinding Safeguards

A number of measures, described below, will be undertaken in order to maintain the blind on this trial. Measures include:

a) Administration of study vaccine in an opaque container.
b) The randomization schedule will be loaded in the EDC system at the beginning of the study. Treatment information will be available only to the unblinded site personnel on a per-subject basis upon randomization of each subject or dispensation of treatment.
c) At each site there will be unblinded personnel who prepare and dispense VAXCHORA and Placebo, and administer the vaccination. All unblinded personnel will agree not to provide any information or documentation to the subjects or blinded staff members that may reveal the treatment assignment. The unblinded personnel will not be involved in any observation, monitoring, or reporting required by the study protocol, other than study vaccine administration, study vaccine accountability and dispensing records.
d) Subjects will be asked not to discuss the taste of the product with other study subjects as appropriate for age and cognitive level.
e) Should any subject or blinded staff member become inadvertently unblinded for any reason, the event will be promptly disclosed to the Investigator so that corrective action can be initiated in consultation with PaxVax. The unblinding sequence of events will be documented and retained as source documents. A protocol deviation will be entered in the eCRF.

5.6 Medical History

Medical history information collected from subjects (or parents or legal guardian) should include (but not be limited to) demographic information, current and past medical conditions, and current medications taken within 30 days of vaccination. The medical history must be documented in the subject's study chart (the on-site source document) prior to study vaccine administration and also recorded on the Medical History electronic case report form (eCRF) and the demographics eCRF. Medications including non-study vaccines must be documented in the source documents as well as on the prior and concomitant medications eCRF and Vaccine History eCRF.

5.7 Physical Examination

A complete physical examination will be performed on subjects during screening.

The examination will include:

Height
Body Weight
Head-Neck

Eyes-Ears-Nose-Throat
Musculoskeletal
Lymph Nodes
Lungs-Chest
Heart
Abdomen
Other body system if applicable

All physical exam findings must be documented in the subject's study chart and also recorded on the appropriate eCRF.

5.8 Vital Signs

Vital Signs collected from subjects during screening and/or at Day 1 and Day 181 (for Placebo crossover subjects) will include blood pressure, pulse rate, respiration and temperature all of which should be recorded when the subject is calm and ready for vital sign assessment in the opinion of the Investigator. Temperature will be measured orally for Cohort 1 (12 to <18 years) and Cohort 2 (6 to <12 years) and oral is preferred for Cohort 3 (2 to <6 years). For Cohort 3 subjects <4, the axillary temperature may be measured if oral is not feasible.

5.9 Pregnancy Testing and Contraception

Female subjects who are of childbearing potential (Cohort 1 and Cohort 2: a female subject is considered of childbearing potential following menarche and until becoming post-menopausal unless permanently sterile) will undergo a urine pregnancy test at screening and prior to each study vaccine administration. This will be done using a urine dipstick. The subject must not receive study vaccine if she has a positive pregnancy test at screening or Day 1, or Day 181 for the Placebo crossover group. Any pregnancies that occur in females who have received a study vaccination must be reported promptly to PaxVax as described in Section 7.7.1.

Female subjects of childbearing potential must also use an acceptable method of contraception from prior to Day 1 through Day 29 and from Day 181 through Day 209 (Placebo crossover subjects). Acceptable methods include highly effective forms of contraception such as combined estrogen and progestogen containing or progestogen-only hormonal contraception associated with inhibition of ovulation, IUD, intrauterine hormone-releasing system, bilateral tubal occlusion, abstinence or vasectomized partner. The Investigator must confirm that contraception methods initiation prior to Day 1 (e.g. hormonal contraception) are considered fully effective prior to vaccination.

5.10 Vaccination

Once Day 1 pre-vaccination procedures and randomization are performed, the study vaccine (VAXCHORA or placebo) will be given by oral administration; subjects will receive nothing by mouth (eat or drink) for 60 minutes prior to and after the study vaccine administration .

Subjects will be asked to swallow the study vaccine in isolation of other subjects and in the presence of the designated unblinded study staff member. The subject will then remain in the clinic for at least a 30-minute observation period which the blinded study staff will monitor the subject for any signs of an acute adverse response to the vaccine.

5.11 Acceptability and Palatability Assessments

Study staff will assess the acceptability of the study vaccine based on the subject's ability to take the study vaccine according to the pharmacy manual. Clinically significant findings immediately after consuming the study vaccine will be reported as adverse events. After completion of the 30-minute observation period, palatability will be assessed by the subject or caregiver and reviewed by blinded study staff using a 5-point Hedonic scale. Further details will be provided in the PXVX-VC-200-006 Manual of Procedures.

5.12 Immune Response Assessments

Blood samples will be collected from all subjects and serum will be tested for vibriocidal antibodies. Table 4 outlines the time points for vibriocidal assessments by subject group.

Table 4: Blood Sample Collection Time Points

Subject Group	Serum Vibriocidal Antibodies (SVA) Assessment with Classical Inaba (Study Days)	Blood Collection for Memory B Cell Test (Study Days)
Cohort 1	1, 11, 29(±2), 91(±7), 181(±7)	1, 91(±7), 181(±7)
Cohort 2, 3	1, 11, 29(±2)	NA (Not Applicable)
Sub-study: Long-Term Follow-up visit for Active Treatment group in Cohort 1	365(±14), 547(±14), 730(±14)	365(±14), 547(±14), 730(±14)

Details regarding sample collection, processing and shipping can be found in the PXVX-VC-200-006 Laboratory Manual.

5.13 Solicited Adverse Events

Solicited adverse events for this study are: abdominal pain, nausea, vomiting, diarrhea, headache, lack of appetite, tiredness, and fever. From Day 1 through Day 8 (for all subjects) and from Day 181 through Day 188 (for Placebo crossover subjects), these adverse events will be solicited from subjects daily in the evening. Before discharge from the clinic on Day 1, all subjects will be trained to complete a memory aid to observe, measure, and record solicited AEs from Day 1 through Day 8. Study staff will review the signs and symptoms recorded on the memory aid with the subject to confirm severity and relationship to study vaccine, and action taken for the AE. The memory aid will be collected as a source document. Solicited adverse events from Day 1 through Day 8 will be recorded on the solicited adverse event eCRF. Symptoms continuing beyond Day 8 will be recorded on the adverse event eCRF. Actions taken for solicited AEs after Day 8 should be noted in the eCRF. Memory aid re-distribution and retraining to collect solicited adverse events from Day 181 through Day 188 (7 days after vaccination) will be done at Day 181 (vaccination) for Placebo crossover subjects. It will be reviewed by the study staff at the Day 191 (10 days after vaccination) visit.

5.14 Adverse Events

All unsolicited adverse events will be collected from vaccination Day 1 through Day 29 for all subjects and from Day 181 through Day 209 for Placebo crossover subjects. Serious adverse events will be collected for the duration of study participation (i.e. starting at Informed Consent through Day 181 for all subjects, additionally through Day 365 for Placebo crossover subjects, and additionally through Day 730 for Cohort 1 sub-study subjects). In Cohort 2 and 3, events occurring after Day 29 may be assessed initially by telephone contact since these cohorts do not have in clinic visits after Day 29. The details of each event will be recorded on the appropriate CRF and in the source document. The reported events may not represent a verbatim accounting by the subject but should reflect the clinical judgment of the study staff. Refer to Section 7 for additional details about evaluating and reporting AEs.

5.15 Prior and Concomitant Medications

Prior and concomitant medications at the screening visit and each subsequently scheduled visit, the details of prior and concomitant medication usage will be collected and recorded on the appropriate eCRF.

Prior and concomitant medications (including non-study vaccines) should be collected from 30 days prior to Day 1 through Day 181 for all subjects, and through Day 365 (6 months after active vaccination) for Placebo crossover subjects, and additionally through Day 730 for Cohort 1 sub-study subjects.

5.16 Prohibited Medications

Subject must not have received or be planning to receive any other licensed vaccines or antibiotics or chloroquine from 14 days prior to Day 1 randomization through 11 days after study vaccine administration. For Placebo crossover subjects, the subjects must not have received any other licensed vaccines or antibiotics or chloroquine 14 days prior to Day 181, and the subject must not be planning to receive any other licensed vaccines or antibiotics or chloroquine through 11 days after active vaccination. Subjects must not have received or be planning to receive any other investigational agents throughout the main study up to Day 181 and placebo crossover up to Day 365 (6 months after active vaccination).

5.17 Emergency Unblinding

The Investigator may obtain a treatment assignment before Day 181 for a study subject only in the case of medical emergency where knowledge of the treatment is necessary for the management of an adverse event. The Investigator will notify the Sponsor (Section 7.7.4) immediately after unblinding, document the reason and circumstances for the unblinding event, and distribute unblinding information only as needed for medical management.

5.18 Protocol Deviations

The Investigator is responsible for conducting the study in accordance with the protocol. Any deviation from the protocol must be documented in the study file. In addition, deviations must be reported to the IRB as applicable. Subject-specific deviations must be recorded in the subject's source documents and in the subject's protocol deviation eCRF. The Sponsor will review all protocol deviations on an ongoing basis and will be responsible for categorizing protocol

deviations as Important Protocol Deviations (IPDs). IPDs may require additional documentation as requested by the Sponsor.

5.19 Completion of Study Participation for Individual Subjects

An individual subject in VAXCHORA treatment groups of Cohort 1, 2, and 3 is considered to complete study participation after completion of the Day 181(±7) visit and completion of any required safety follow-up. An individual subject in the Placebo crossover groups of Cohort 1, 2, and 3 will be offered VAXCHORA at the Day 181 visit and is considered to complete study participation after completion of the Day 365 (6 months after active vaccination) visit if the placebo crossover subject opts to receive VAXCHORA. The placebo crossover subject who opts not to receive VAXCHORA is considered to complete at Day 181. An individual in the long-term follow-up group of the Cohort 1 sub-study is considered to complete study participation after completion of the Day 730 (±14) visit and completion of any required safety follow-up.

5.20 Early Discontinuation

An enrolled (randomized) subject is considered to undergo Early Discontinuation if they stop study participation before the study completion visit day of each group.

An enrolled subject may voluntarily withdraw consent for further participation at any time before the day of study completion. The Investigator will request (but cannot require) such subjects to provide the reason(s) for withdrawal of consent and to undergo an Early Discontinuation visit.

In addition, the Investigator, at his or her discretion, may withdraw a subject from further participation in the study. Criteria for withdrawal by the Investigator include:

- Noncompliance with the protocol
- Lost to followup: lost to followup requires documentation of at least 3 unsuccessful attempts to contact subjects. Lost to followup will be determined after subject's projected last visit day.
- Adverse event
- Other reason(s) which, in the opinion of the Investigator, indicates that continued participation in the study is not in the best interest of the subject

5.21 Subject Replacement

Subjects who undergo Early Discontinuation after randomization and before vaccination may be replaced at the Sponsor's discretion. Subjects who undergo Early Discontinuation after vaccination will not be replaced. Neither subject numbers nor randomization numbers will be reused.

5.22 Study Completion

The study is planned to be completed after all subjects have completed the planned visits (or Early Discontinuation), all necessary safety follow-up has been completed, and all data queries are resolved, the eCRFs are signed by the Investigator, and the data are locked. The Sponsor reserves the right to terminate the study prior to the planned study completion.

6 STUDY PROCEDURES BY VISIT

The overall summary of evaluations by visit is given in the Schedule of Events in Appendix A. All visits are relative to the day of vaccination, Day 1. Acceptable time windows for the visit schedule are indicated.

6.1 Study Visits

6.1.1 Screening Visit (-30 Days to Day 1)

The following will take place during the visit:

- Informed Consent
- Medical History
- Prior and concomitant medications
- Physical Exam
- Vital Signs
- Urine Pregnancy test (females of childbearing potential in Cohorts 1 and 2)
- Inclusion/Exclusion Criteria

6.1.2 Day 1

The Day 1 Visit must occur no more than 30 days after the initial Screening Visit. All eligible, consented/assented, and randomized subjects are vaccinated at this visit.

The following will take place during the visit and ***prior to*** study vaccine administration:

- Updated Medical History
- Directed Physical Examination, if indicated by updated medical history
- Blood Collection (**pre-dose**) for:
  - Serum Vibriocidal Antibody (SVA)
  - Memory B cells for Cohort 1 only (optional)
- Urine Pregnancy test (females of childbearing potential) Updated prior/concomitant medications
- Vital Signs
- Confirmation Inclusion/Exclusion Criteria are still met
- Randomization

Study Vaccine Administration:

Subjects will take nothing by mouth (food or water) for 60 minutes before and after consumption of study vaccine.

The following will take place during the visit and ***after*** consumption of study vaccine:

- In-clinic evaluation of acute side effects at least 30 minutes post-vaccination
- Acceptability/Palatability evaluation
- Adverse event evaluation
- Memory Aid - distribution and training

6.1.3 Day 11

The following will take place during the visit:

- Memory Aid-collect and review
- Adverse event evaluation including SAE
- Blood Collection for:
    - Serum Vibriocidal Antibody (SVA)
- Updated concomitant medications

6.1.4 Day 29 (±2)

The following will take place during the visit**:**

- Adverse event evaluation including SAE
- Updated concomitant medications
- Blood collection for:
    - Serum Vibriocidal Antibody (SVA)

6.1.5 Day 91 (± 7):

a) Cohort 1

The following will take place at this visit:

- Serious adverse event evaluation
- Updated concomitant medications
- Blood Collection for:
    - Serum Vibriocidal Antibody (SVA)
    - Memory B cells (optional)

b) Cohort 2 and 3

Telephone contact is required to assess the following:

- Serious adverse event evaluation
- Updated concomitant medications

6.1.6 Day 181 (± 7 days): Unblinded

a) Cohort 1

The following will take place at this visit prior to unblinding:

- Serious adverse event evaluation
- Updated concomitant medications
- Blood Collection for:
    - Serum Vibriocidal Antibody (SVA)
    - Memory B cells (optional)

The following will take place at this visit after unblinding:

- Consent for sub-study (for the Active Treatment Group only)

b) Cohort 2 and 3

The following will take place at this visit prior to unblinding:

- Serious adverse event evaluation
- Updated concomitant medications

c) Placebo Crossover Groups only (Cohort 1, 2, 3): optional

VAXCHORA will be offered to the subjects who were administered Placebo on Day 1after unblinding.

The following will take place during this visit ***prior to*** Vaccination:

Subjects will take nothing by mouth (food or water) for 60 minutes before and after vaccination.

- Urine Pregnancy Test for Cohort 1 and 2
- Vital Signs

The following will take place during the visit and ***after*** vaccination:

- In-clinic evaluation of acute side effects for at least 30 minutes post-vaccination
- Acceptability/Palatability evaluation
- Adverse event evaluation
- Memory Aid - distribution and training

6.1.7 Day 191 (10 days after active vaccination): for Placebo crossover group only

The following will take place during the visit:

- Memory Aid - collection and review
- Adverse event evaluation including SAE
- Updated concomitant medications

6.1.8 Day 209 (±2) (28 days after active vaccination): for Placebo crossover group only

The following will take place during the visit:

- Adverse event evaluation including SAE
- Updated concomitant medications

6.1.9 Day 271 (±7) (90 days after active vaccination): for Placebo crossover group only

Telephone contact is required to assess the following:

- Serious adverse event evaluationUpdated concomitant medications

6.1.10 Day 365 (±7) (6 months after active vaccination): for Placebo crossover group only

Telephone contact is required to assess the following:

- Serious adverse event evaluation
- Updated concomitant medications

6.1.11 Day 365 (± 14 days) for long term follow-up group (sub-study for Cohort 1 only)

The following will take place at this visit:

- Serious adverse event evaluation
- Updated concomitant medications
- Blood Collection for:
    - Serum Vibriocidal Antibody (SVA)
    - Memory B cells (optional)

6.1.12 Day 547 (± 14 days) for long term follow-up group (sub-study for Cohort 1 only)

The following will take place at this visit:

- Serious Adverse Event evaluation
- Updated concomitant medications
- Blood Collection for:

- Serum Vibriocidal Antibody (SVA)
- Memory B cells (optional)

6.1.13 Day 730 (± 14 days) for long term follow-up group (sub-study for Cohort 1 only)

The following will take place at this visit:

- Serious adverse event evaluation
- Updated concomitant medications
- Blood Collection for:
  - Serum Vibriocidal Antibody (SVA)
  - Memory B cells (optional)

6.2 Early Discontinuation Visit

All subjects who discontinue study participation before the planned study completion Day 181 visit will undergo an Early Discontinuation visit.

If the visit occurs before Day 11, the following will be conducted:

- Review Solicited AEs, unsolicited AEs, and Serious Adverse Events (SAEs)
- Update concomitant medications associated with new or ongoing AEs and any SAEs
- Blood Collection for: Serum Vibriocidal Antibody (SVA)

If the visit occurs after Day 11 and before Day 29, the following will be conducted:

- Review unsolicited AEs, and Serious Adverse Events (SAEs)
- Update concomitant medications associated with new or ongoing AEs and any SAEs
- Blood Collection for: Serum Vibriocidal Antibody (SVA)

If the visit occurs after Day 29, the following will be conducted:

- Review Serious Adverse Events (SAEs)
- Update concomitant medications associated with any SAEs
- Blood Collection for:
  - Serum Vibriocidal Antibody (SVA)
  - Memory B cells (optional): for Cohort 1 only

6.3 Unscheduled Visits

Additional study procedure(s) may be conducted at an unscheduled visit as needed. Examples include repeat specimen collection and additional safety follow-up for an adverse event.

7 SAFETY

7.1 Definitions

7.1.1 Adverse Event

An Adverse Event (AE) is any untoward medical occurrence in a study participant, regardless of the suspected causal relationship with study vaccine.

The definition of an AE includes:

- A new-onset symptom or disease
- An exacerbation of a pre-existing symptom or disease (worsening in frequency or intensity)
- A new-onset laboratory abnormality considered by the Investigator to be clinically significant
- A new-onset symptom or disease that occurs as a result of a protocol-specified procedure

The definition of an AE does **not** include:

- A pre-existing symptom or disease that does not worsen during the study (even if first disclosed by the subject after the start of the study)
- A medical or surgical intervention such as surgery, endoscopy, tooth extraction, or transfusion (although the condition leading to the procedure or a complication from the procedure may be an AE)
- An uncomplicated pregnancy
- A dosing error without any resulting signs or symptoms
- Any other situation where an untoward medical occurrence has not occurred (e.g. hospitalization for cosmetic elective surgery or social admissions)

The Investigator will attempt to establish a diagnosis based on signs, symptoms, and other clinical information. Whenever possible, the Investigator will report an AE as a diagnosis rather than one or more signs or symptoms. If a clinically significant laboratory abnormality meets the definition of an AE, a diagnosis or clinical signs and symptoms rather than the abnormal laboratory finding should be reported if possible. If no diagnosis is known and clinical signs and symptoms are not present, but the laboratory abnormality is clinically significant by itself, then it should be reported as the AE.

7.1.2 Solicited Adverse Event

A solicited adverse event (solicited AE) is a protocol-specified AE about which the Investigator or designee proactively asks the subjects during a protocol-specified time period. Solicited adverse events for this study are: abdominal pain, nausea, vomiting, diarrhea, headache, lack of appetite, fever, tiredness.

7.1.3 Unsolicited Adverse Event

An unsolicited adverse event (unsolicited AE) is an AE that is spontaneously reported by the subject or discovered by the Investigator.

7.1.4 Serious Adverse Event

An SAE is an AE (either solicited or unsolicited) which meets any of the following criteria:

- Results in death
- Is life-threatening
- Requires hospitalization or prolongs an existing hospitalization
- Results in a persistent clinically significant disability or incapacity
- Is a congenital anomaly or birth defect
- Requires medical or surgical intervention to prevent one of the above outcomes
- Important medical event

The Investigator will evaluate all AEs for seriousness using the above criteria.

"Life-threatening" means that in the opinion of the Investigator, the subject was at immediate risk of death from the event as it occurred. It does not mean that the event might have caused death had it occurred in a more severe form.

Hospitalization for observation or for elective treatment of a pre-existing condition that did not worsen during the study is not considered an SAE.

Important medical events may be considered serious at the discretion of the Investigator.

These seriousness criteria also apply to the Study Stopping Rules in Section 7.10.

7.2 Severity Grading

The Investigator will grade all adverse events for severity. Adverse events listed in the Toxicity Grading Scale in Appendix B will be graded according to the criteria in the table. Adverse events not listed in the Toxicity Grading Scale will be graded as follows:

- Mild (Grade 1) – No interference with activity
- Moderate (Grade 2) – Some interference with activity
- Severe (Grade 3) – Significant; prevents daily activity
- Potentially Life-Threatening (Grade 4) – ER visit or hospitalization
- Fatal (Grade 5)

These severity grades also apply to the Study Stopping Rules in Section 7.10.

7.3 Causality Assessment

The Investigator will assess all AEs, including solicited AEs, for causality (relationship to study vaccine), assigning one of these three categories: Not Related, Possibly Related, and Probably Related.

An AE will be considered "Not Related" to study vaccine if **any** of the following conditions are met:

- An unreasonable temporal relationship between administration of the study vaccine and the onset of the AE (e.g., the event occurred either before, or too long after administration of the study vaccine for it to be considered related);
- A causal relationship between the study vaccine and the AE is biologically implausible (e.g. injury as a passenger in an automobile accident);
- A clear alternative causality for the AE is present (e.g. typical adverse reaction to a concomitant medication).

An AE will be considered "Possibly Related" if there is a reasonable possibility that the AE may have been caused by the study vaccine.

An AE will be considered "Probably Related" if there is evidence that the AE was caused by the study vaccine.

7.4 Follow-up of Adverse Events

The Investigator must follow all AEs until resolution, until the condition stabilizes or is no longer clinically significant, or until the subject is lost to follow-up. The requirement for follow-up includes solicited adverse events that continue past Day 8.

The Investigator is responsible for ensuring the conduct of any supplemental investigations considered necessary to evaluate the AE. These may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.

In the event of a non-fatal SAE, subjects will be instructed to contact the Investigator (or designee) immediately. All subjects experiencing an SAE will be evaluated by the Investigator or designee as soon as is feasible following the report of the SAE by the subject. In the event of a fatal SAE, the Investigator must provide the Sponsor with any available post-mortem findings, including histopathology.

Additionally, the Sponsor may request that the Investigator perform or arrange for the conduct of supplemental investigations for one or more AEs.

7.5 Reporting of Adverse Events

7.5.1 Reporting Periods

The reporting period for SAEs begins at the time of informed consent and continues for the duration of study participation (Section 5.14). The reporting period for solicited AEs begins after

study vaccine administration on Day 1 and continues through Day 8. The reporting period for solicited AEs for the Placebo crossover subjects begins after VAXCHORA administration on Day 181 and continues through Day 188.The reporting period for all other AEs begins after study vaccine administration on Day 1 and continues through Day 29. The reporting period for all other AEs for the Placebo crossover begins after VAXCHORA administration on Day 181 and continues through Day 209. AEs that correspond to solicited AE terms and occur after Day 8 or Day 188 (for Placebo crossover subjects) will be reported as unsolicited AEs.

7.5.2 Documentation

The Investigator or designee will document all AEs in the subject's source documents and enter all unsolicited AEs or solicited AEs continuing after Day 8 in the adverse event eCRF within 7 days of awareness.

All unsolicited AEs should include:

- Event term
- Start and stop date
- Severity
- Seriousness (Yes/No) and Seriousness criteria met if applicable
- Relationship to study vaccine
- Action taken in response to the AE

7.6 SAE Reporting

The Investigator or designee must report all SAEs to the Sponsor Medical Monitor within 24 hours of their awareness of the event by filling out the paper SAE Form and send to [redacted]. The Investigator or designee must also enter SAEs in the adverse event eCRF.

The SAE Form should be completed as thoroughly as possible and signed by the Investigator or designee before reporting to the Sponsor Medical Monitor. The SAE Form must include an assessment of causality and should include a preliminary diagnosis if possible.

In order to avoid delays in initial reporting, additional information regarding the SAE may be provided as a follow-up report. The Investigator may also modify the diagnosis, seriousness, and/or causality assessment based on this information.

The Sponsor Medical Monitor will notify the Investigator of SAEs that meet criteria for expedited reporting to regulatory authorities. The Investigator is responsible for notifying the applicable IRB of these events and adhering to any other applicable local reporting requirements.

The Sponsor will report adverse events to FDA in accordance with 21 CFR 312.32. Specifically, the Sponsor will report unexpected fatal or life-threatening suspected adverse reactions no later than 7 days after initial receipt of the information, and serious and unexpected suspected adverse

reactions (SUSARs) no later than 15 calendar days after determining that the information qualifies for expedited reporting.

7.7 Other Events Requiring Immediate Reporting

7.7.1 Pregnancy

The Investigator or designee must submit the Pregnancy Report Form to [redacted] within 24 hours of their awareness of the pregnancy. All pregnancies will be followed to outcome. Additional information regarding the pregnancy may be provided as a follow-up report.

An uncomplicated pregnancy is not considered an AE. Complications of pregnancy may qualify as AEs or SAEs and would therefore be documented and reported as specified above.

7.7.2 Dosing Errors

The Investigator or designee must report any error in the dosing of study vaccine to the Sponsor Medical Monitor in a blinded manner, and report any unblinding information to the unblinded CRA within 24 hours of their awareness of the error. Additional information regarding the dosing error may be provided as a follow-up report. A dosing error without signs or symptoms is not considered an AE, but is a protocol deviation and may be determined to be an IPD.

7.7.3 Early Discontinuation for Safety Reasons

The Investigator or designee must report any early discontinuation for safety reasons to the Sponsor Medical Monitor within 24 hours of discontinuation (Section 7.6). Additional information regarding ongoing adverse events may be provided as a follow-up report.

7.7.4 Emergency Unblinding for Safety Reasons

The Investigator or designee must report emergency unblinding to the Sponsor's Medical Monitor in a blinded manner within 24 hours of unblinding. The Investigator or designee must fill out the Unblinding Request Form and send to [redacted] and can discuss any unblinding information with the unblinded CRA. Additional information regarding the unblinding, excluding the actual treatment assignment, may be provided as a follow-up report.

7.8 Medical Monitor

The Sponsor Medical Monitor (SMM) will provide safety oversight for the study. The SMM or designee will review blinded study data and assess causality of SAEs on an ongoing basis.

Name: [redacted]
Title: [redacted]
Address: [redacted]
Redwood City, [redacted]
Telephone: [redacted]
Fax: [redacted]
Email: [redacted]

7.9 Safety Monitoring Committee (SMC)

A Safety Monitoring Committee (SMC) will be appointed to conduct periodic review of all available interim, blinded safety data from simultaneous enrollment from all age cohorts every 6 months while study activities are ongoing. The SMC will be composed of two independent physicians and one independent statistician. The SMC charter will outline the specific data to be reviewed and the communication plan.

7.10 Study Stopping Rules

Enrollment will be stopped for any of the following:

1. Any death or SAE experienced by a subject, regardless of causality;
2. One or more subjects with a Grade 3 (severe) or greater AE assessed as possibly or probably related to the study vaccine.

The investigator or designee must suspend enrollment and notify the Medical Monitor at [redacted] within 24 hours of becoming aware of an event that meets either of the above rules. No specific notification form is required. Enrollment may be resumed following review of available safety data by the Sponsor's Medical Monitor and Safety Monitoring Committee and after the Sponsor's Medical Monitor has notified the Investigator that enrollment may resume.

For further information regarding Safety procedures, please refer to the PXVX-VC-200-006 Safety Management Plan (SMP) and PXVX-VC-200-006 SMC Charter.

8 DATA HANDLING

8.1 Source Documentation

The Investigator must maintain source documentation of all study conduct and data and observations relevant to the study. This source documentation includes, but is not limited to, ICFs, original medical records, progress notes from the Investigator and study staff, laboratory reports, memory aids for solicited adverse events, and documentation of study vaccine accountability. The investigator may keep electronic source documentation per Sponsor's request.

8.2 Case Report Forms

This study will employ electronic Case Report Forms (eCRFs) provided by the Sponsor. Certain clinical information requested in this protocol will be recorded on these eCRFs. The Investigator is responsible for the adequacy and accuracy of all data entered on the eCRFs. The Investigator is also responsible for signing all eCRFs, after which they will be locked to prevent further data entry or modification.

For further information on eCRFs, please refer to the PXVX-VC-200-006 CRF Completion Guidelines. For further information regarding data handling, please refer to the PXVX-VC-200-006 Data Management Plan (DMP).

8.3 Retention of Study Documentation

The Investigator must make study data accessible to the monitor, other authorized representatives of PaxVax (and/or its designee), and regulatory agencies (e.g., FDA) inspectors upon request. A file for each subject must be maintained that includes the signed ICF and all source documentation related to that subject. The Investigator must ensure the reliability and availability of source documents from which the information on the eCRF was derived. Investigators are required to maintain all study documentation, including copies of eCRFs, ICFs, and adequate records for the receipt and disposition of all study medications, for a period of 2 years following the FDA or other regulatory approval of VAXCHORA, or 2 years after clinical development of VAXCHORA is discontinued, unless a longer period is required by applicable law or regulation. The Investigator will destroy study documentation only upon instruction by the Sponsor and must notify the Sponsor upon completion of such destruction. Subject identity information will be maintained for 15 years unless a longer period is required by applicable law or regulation.

8.4 Data Monitoring

The Sponsor or designee will monitor completed eCRFs against source documentation at regular intervals throughout the study to verify adherence to the protocol, completeness, accuracy, and consistency of the data, and adherence to local regulations on the conduct of clinical research. The Investigator must make source documentation accessible to the Sponsor or designee as needed to verify the information in the eCRFs. The Investigator agrees to cooperate with the Sponsor or designee to ensure that any problems detected in the course of data monitoring are resolved.

8.5 Laboratory Data

This study will employ electronic transfers of external laboratory data generated from clinical specimens collected by the Investigator. The Investigator is responsible for the adequacy and

accuracy of data associated with collection of these specimens. The Sponsor or designee is responsible for the adequacy and accuracy of the data generated by external laboratories.

8.6 Audit Compliance

The Investigator must permit the Sponsor and/or designee, regulatory agencies, and/or the IRB direct access to facilities and study documentation for the purpose of auditing study conduct. Direct access includes examining, analyzing, verifying, and reproducing any records and reports that are needed for the evaluation of the study.

9 STATISTICAL ANALYSIS

9.1 Sample Size Calculation

Assuming that the true seroconversion rate among 12 to <18-year-olds is 92.4% or higher, the sample size of 143 evaluable vaccinees for that age cohort affords 93.3% power to demonstrate that the seroconversion rate within the group is non-inferior to the 94% rate observed in the 2687 adult subjects assessed in the PaxVax lot-consistency trial, PXVX-VC-200-004. The sample size allows for up to 5% of subjects to be not evaluable for the primary immunogenicity analyses in the two older cohorts (ages 6 to < 18). Based on the inevaluable rate observed to date, it is estimated that approximately 30% of subjects in the youngest cohort (ages 2 to < 6) will be inevaluable, mostly due to lack of consumption of the full dose. As defined in the alpha-spending strategy described in Section 9.5.1, non-inferiority implies that the lower bound on the two-sided 96.7% confidence interval on the seroconversion rate among 12 to <18-year-olds is within 10 percentage points of the adult rate. Making similar assumptions about the true seroconversion rates in the other two pediatric age cohorts, and assuming that the rates in the three age cohorts are independent of one another, the overall power for demonstrating the non-inferiority of all three age cohorts is $(93.3\%)^3 = 81\%$.

To meet the other primary objective, the lower bound of the two-sided 98.3% confidence interval on the cohort-specific seroconversion rate must equal or exceed 70%. Under the same assumptions as above – in particular, assuming the true seroconversion rate within an age cohort is at least 92.4% – 143 evaluable vaccinees provide greater than 99.9% power to establish that the lower bound on the cohort-specific rate is at least 70%. Power is still greater than 99.9% when requiring the lower bound on all three cohorts to be 70% or greater.

The overall power of meeting both primary objectives in all three age cohorts can be approximated by multiplying the power of meeting the non-inferiority objective by the power of meeting the 70% lower bound objective: 81% x 99.9% ≈ 81%. Note that the calculations above rely on the assumption that seroconversion rate in one cohort is completely independent of the rate in another. Since that assumption is conservative, it is likely that the true power of the trial is higher than 81%.

Given a total of 510 subjects receive VAXCHORA, there is a 99% chance that an uncommon AE - one expected to occur in only 1% of vaccinees - will be observed at least once during the trial.

9.2 Treatment Period

The main study consists of a screening period of 30 days, Day 1 vaccination, an observation period from Day 1 to Day 29, and a follow-up period through Day 181. Placebo crossover has an optional active vaccination at Day 181 after unblinding, with a 6 month post vaccination follow-up period. Cohort 1 has an optional sub-study consisting of a long-term follow-up period through Day 730.

9.3 Treatment Groups

- Subjects in the active treatment group in each Cohort at Day 1 will receive VAXCHORA.

  Cohort 1: 12 to <18 years (Active treatment group: n=150)
  Cohort 2: 6 to <12 years (Active treatment group: n=150)

Cohort 3: 2 to < 6 years (Active treatment group: n=210)

- Subjects in the Placebo crossover group in each Cohort at Day 181 (±7) will be offered VAXCHORA.

Cohort 1: 12 to <18 years (Placebo crossover group: n=25)
Cohort 2: 6 to <12 years (Placebo crossover group: n=25)
Cohort 3: 2 to < 6 years (Placebo crossover group: n=35)

9.4 Safety Analysis

Safety will be assessed primarily through the evaluation of solicited and unsolicited AEs.

9.4.1 Solicited AEs

Solicited AEs recorded during Days 1-8 will be summarized. Specifically, a table will summarize the percent of subjects within each treatment group who experience each solicited adverse event at least once. A second table will present a similar summary constrained to solicited AEs of moderate severity or higher. An additional analysis will present the minimum, median and maximum number of days that subjects in each treatment group experience each type of solicited AE. The information regarding the incidence and severity of solicited AEs reported by Placebo crossover subjects between Days 181 and 188 will be summarized and listed.

9.4.2 Unsolicited AEs

Unsolicited AEs recorded through Day 29 will be summarized by treatment group, system organ class, preferred term and severity. Unsolicited AEs collected from Days 181 to 209 will be summarized separately for Placebo crossover subjects.

SAEs recorded through Day 181 or Day 365 (for Placebo crossover subjects) or Day 730 (for sub-study subjects) will be presented.

9.5 Immunogenicity Analysis

9.5.1 Primary Immunogenicity Analyses

Seroconversion will be defined as a 4-fold or greater rise in SVA over Day 1. Each rate will be accompanied by a 95% confidence interval (CI) calculated using the Wilson method (Agresti 1998).

1. The seroconversion rate between Day 1 and Day 11 for vaccinees in each of the 3 age cohorts will be compared to the seroconversion rate for vaccinees between the ages of 18 and 45 who participated in the lot consistency study PXVX-VC-200-004 by calculating the difference between the two rates and computing a 96.7% confidence interval for this difference.

2. For each of the 3 age cohorts, the proportion of vaccinated subjects who experience a 4-fold or greater increase in serum vibriocidal titer between Day 1 and Day 11 will be calculated, and a 98.3 % confidence interval on this proportion of seroconverters will be computed.

Acceptance Criteria for Primary Analyses

1. The lower bound of the two-sided 96.7% confidence interval on the difference in seroconversion rates between children and adults must be greater than -10 percentage points.

2. The lower bound of the two-sided 98.3% confidence interval on the proportion of vaccinees who seroconvert between Day 1 and Day 11 must equal or exceed 70%.

Alpha-Spending Strategy

In order to ensure that the total Type I error for the study is capped at alpha = 0.05, 2/3 of the alpha will be allotted to the primary objective of establishing non-inferiority relative to adults, and 1/3 of the alpha will be allotted to the primary objective of demonstrating that the seroconversion rate equals or exceeds 70%. The two primary objectives will be evaluated independently, and within each primary objective, testing in the different age cohorts will proceed sequentially beginning with the data for the 12 to <18 age cohort, as follows:

1. Non-inferiority: the analysis of non-inferiority between vaccinees in the 12-18 age cohort and adults between the ages of 18 and 45 will be conducted first. Formal analysis of non-inferiority in the 6 to <12 age cohort will be performed only if the lower bound of the two-sided 96.7% confidence interval on the difference between the seroconversion rates for children in the 12 to <18 age cohort and adults equals or exceeds the pre-specified acceptance criterion. Formal evaluation of non-inferiority in the 2 to <6 age cohort will occur only if the acceptance criterion is met for both the 12 to <18 and 6 to <12 age cohorts. This strategy maintains the overall Type I error for the primary objective of non-inferiority at alpha = 0.033.

2. Seroconversion Rate: the analysis of the seroconversion rate among vaccinees in the 12 to <18 age cohort will be conducted first. Formal analysis of the acceptability of the seroconversion rate in the 6 to <12 age cohort will be performed only if the lower bound of the two-sided 98.3% confidence interval on the seroconversion rate in the 12 to <18 age cohort meets the pre-specified acceptance criterion. Formal evaluation of the acceptability of the seroconversion rate in the 2 to <6 age cohort will occur only if the acceptance criterion is met for both the 12 to <18 and 6 to <12 age cohorts. This strategy maintains the overall Type I error for the primary objective concerning the magnitude of the seroconversion rate at alpha =0.017.

9.5.2 Secondary Analyses

For vaccinees in the age cohort 12 to <18, the point estimate of the seroconversion rate for vaccinees as well as the cumulative seroconversion rate will be computed at the time points Day 29, Day 91 and Day 181, and a 95% confidence interval will be constructed for each proportion. Pointwise seroconversion rates, cumulative seroconversion rates, and confidence intervals for placebo recipients in the age cohort 12 to <18 will be computed at Day 11, Day 29, Day 91 and Day 181. For vaccinees in the age cohorts 2 to <6 and 6 to <12, pointwise and cumulative seroconversion rates and corresponding confidence intervals will be calculated for Day 29. Pointwise seroconversion rates, cumulative seroconversion rates, and confidence intervals for placebo recipients in the age cohorts 2 to <6 and 6 to <12 will be computed at Day 11 and Day 29.

For vaccinees and placebo recipients in the age cohort 12 to <18, the GMT and 95% confidence interval will be computed for Day 1, Day 11, Day 29, Day 91 and Day 181, and the GMFI in each treatment group will be calculated for Day 11, Day 29, Day 91 and Day 181. For vaccinees and placebo recipients in the age cohorts 2 to <6 and 6 to <12, GMTs and 95% confidence intervals will be calculated for Day 1, Day 11 and Day 29, and GMFIs and corresponding confidence intervals will be calculated for Day 11 and Day 29. For participants in the sub-study, the GMT and 95% confidence interval and GMFI with 95% confidence interval will be calculated for Days 365, 547 and 730. Number of subjects with SVA titers less than the LLOQ will also be summarized for each collection timepoint.

9.5.3 Memory B Cell Concentration Analyses

In the main study, anti-O1 LPS memory B cells will be assessed at Day 1 (prior to vaccination), at Day 91, Day 181 for a subset of subjects in Cohort 1 and at Days 365, 547, 730 for subjects in the active treatment group of Cohort 1 for the sub study. Point estimates of median percentage as well as median fold rise Day 1 to Day 91, Days 181, 365, 547, 730 will be presented.

9.6 Interim Analysis

After the last subjects enrolled in Cohorts 1 and Cohort 2 have completed their Day 181 visit, a preliminary immunogenicity and safety analysis may be performed and report written to facilitate a regulatory submission. Due to the unblinding of subjects individually at Day 181, the subjects in Cohorts 1 and 2 will have already been unblinded prior to the analysis and the analysis will not affect the blind of the Cohort 3 subjects. The results of these analyses are not expected to alter the course of the trial.

10 ADDITIONAL INFORMATION

10.1 Ethical Conduct of the Study

The study will be performed in accordance with the protocol and consistent with ICH Good Clinical Practice (GCP) Guidelines and applicable local regulatory requirements and laws.

10.2 IRB Oversight

The study (protocol, informed consent form, recruiting materials, and any documents seen by the subject) will be reviewed and approved by an IRB appropriate to each study site. Subjects will not be recruited, consented, screened, or enrolled until the IRB has approved the required documentation. In addition, the IRB will review amendments to the protocol before their implementation.

The Investigator will retain all correspondence with the IRB in the regulatory binder and forward copies of all IRB approvals to the Sponsor.

10.3 Informed Consent and Assent

The Sponsor will provide a master parent/guardian Informed Consent Form (ICF) template to each site for development of a site-specific parent/guardian ICF. The Sponsor will also provide informed assent form (IAF) templates to each site for development of site-specific and age-appropriate assent forms.

All site-specific ICFs and IAFs must be approved by the Sponsor and the IRB and must be in compliance with ICH GCP, local regulatory requirements and legal requirements. The Sponsor will advise the Site of required changes to the master ICF and IAF templates during the course of the study.

The Investigator will ensure that each potential study participant is fully informed about the nature and objectives of the study and possible risks associated with participation. Before informed consent is obtained, the Investigator, or a qualified person designated by the investigator, will provide the potential study participant with ample time and opportunity to inquire about the details of the trial, and will answer all relevant questions to the potential study participant's satisfaction. The potential study participant will then decide whether or not to participate in the trial. The Investigator, or a qualified person designated by the Investigator, will obtain written informed consent from each study participant before any study-specific activity is performed.

The Investigator will retain the original and any amended signed and dated ICFs and IAFs at the study site and provide a copy to each study participant.

10.4 Subject Confidentiality

The Investigator will ensure that each subject's anonymity is maintained. On eCRFs and other documents submitted to the Sponsor and/or its designee, subjects must be identified by subject number only. For documents that are not for submission to the Sponsor and/or its designee (e.g., signed ICFs), the Investigator must maintain these documents securely and in compliance with Federal regulations and ICH GCP Guidelines.

10.5 Compensation for Injury

The Sponsor will adhere to local regulations and guidelines regarding clinical trial compensation to subjects whose health is adversely affected by taking part in the study. The applicable policy for compensation for injury will be described in the master ICF template.

10.6 Clinicaltrials.gov

For purposes of reporting to clinicaltrials.gov, the Sponsor is the responsible party and will provide information regarding this study in accordance with applicable regulations.

10.7 Public Disclosure and Publication Policy

All publication rights are delineated in the Clinical Study Agreement. Sponsor or its designee shall have the right to publish or otherwise publicly disclose the information contained in or related to the Study Vaccine, the Study Data, or other Confidential Information in any form without the written consent of Site, the Principal Investigator or any other person. Each of the Sites and the Principal Investigator further agrees that Sponsor shall have the exclusive right to commercialize any products or services that are based upon, or derived from the Study Vaccine, the Study Data, or other Confidential Information.

10.8 Amendments

The protocol may be amended only by the Sponsor. Agreement from the Investigator must be obtained for all protocol amendments and amendments to the Informed Consent document. The IRB must generally be informed of all amendments prior to implementation. In addition, the Investigator must obtain IRB approval for any amendments likely to affect the safety of study participants prior to implementation.

The Investigator or Sponsor may implement an amendment prior to IRB notification or approval only in order to eliminate an apparent immediate hazard to study participants. In that event, the Investigator must notify the IRB and Sponsor in writing within 7 calendar days after the implementation. Amendments, including descriptions and rationales, will be documented in this section of the protocol. The Investigator must send a copy of the approval letter from the IRB to PaxVax (or its designee).

10.9 Annual Progress Report

PaxVax will submit a summary of the progress of the trial to the IND on file with the FDA once a year. In addition, the Investigator is responsible for submitting progress reports at least annually to the IRB and IBC that approved the study. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, SAEs and serious adverse reactions, other problems, and amendments.

10.10 End of Study Report

The Investigator will notify their IRB and IBC of the end of the study and will submit a final report using the forms and/or format specified. The end of the study is defined as the last subject's last visit. In case the study is ended prematurely, the Investigator will notify the IRB and IBC, including the reasons for the premature termination.

APPENDIX A: SCHEDULE OF EVENTS

COHORT 1 12 to <18 years / Study Day:	Screening	1	11	29	91	181	Early D/C	181[PC]	191[PC]	209[PC]	271[PC] Phone FU	365[PC] Phone FU	365	547	730
Window (days):	-30	0	0	±2	±7	±7	n/a	±7	0	±2	±7	±7	±14	±14	±14
Informed Consent/Assent	X[1]					X									
Medical History	X[1]	X[2]													
Inclusion/Exclusion	X[1]	X													
Physical Exam	X[1]	X[2]													
Vital Signs	X[1]	X						X[PC]							
Con Med Evaluation	X[1]	X[2]	X	X	X	X	X	X[PC]	X[PC]	X[PC]	X[PC]	X[PC]	X	X	X
Urine pregnancy test [d]	X[1]	X						X[PC]							
Vaccination		**X**						X[PC]							
Acceptability/Palatability		X						X[PC]							
Memory Aid [a]		X	X				X[b]	X[PC]	X[PC]						
Solicited AEs		X					X[b]	X[PC]							
Adverse Event Evaluation		X	X	X	X	X	X	X[PC]	X[PC]	X[PC]	X[PC]	X[PC]	X	X	X
SVA		X	X	X	X	X	X						X	X	X
Blood collection for Memory B cell test		X			X	X	X						X	X	X

X = All Subjects; PC = Placebo crossovers only; D/C = discontinuation; SVA = serum vibriocidal antibodies

[1] May be conducted at Day 1

[2] Updated as needed

[PC] placebo crossover subjects are offered VAXCHORA

[a] All subjects: issued at Day 1, reviewed at Day 11

[b] Only if between Day 1 and Day 11 (All subjects)

[d] for females of child-bearing potential

181[PC]: Placebo crossover Vaccination (Day 1)

191[PC]: Placebo crossover 10 days after Vaccination

209[PC]: Placebo crossover 28 days after Vaccination

271[PC]: Placebo crossover 90 days after Vaccination

365[PC]: Placebo crossover 180 days after Vaccination

COHORT 2 6 to <12 years	Study Day:	Screening	1	11	29	91 Phone FU	181	Early D/C	181[PC]	191[PC]	209[PC]	271[PC] Phone FU	365[PC] Phone FU
Window (days):		-30	0	0	±2	±7	±7	n/a	±7	0	±2	±7	±7
Informed Consent/Assent		X[1]											
Medical History		X[1]	X[2]										
Inclusion/Exclusion		X[1]	X										
Physical Exam		X[1]	X[2]										
Vital Signs		X[1]	X						X[PC]				
Con Med Evaluation		X[1]	X[2]	X	X	X[c]	X	X	X[PC]	X[PC]	X[PC]	X[PC]	X[PC]
Urine pregnancy test [d]		X[1]	X						X[PC]				
Vaccination			**X**						X[PC]				
Acceptability/Palatability			X						X[PC]				
Memory Aid [a]			X	X				X[b]	X[PC]	X[PC]			
Solicited AEs			X					X[b]	X[PC]				
Adverse Event Evaluation			X	X	X	X[c]	X	X	X[PC]	X[PC]	X[PC]	X[PC]	X[PC]
SVA			X	X	X			X					

X = All Subjects; PC = Placebo crossovers only; D/C = discontinuation; SVA = serum vibriocidal antibodies

[1] May be conducted at Day 1

[2] Updated as needed

[PC] placebo crossover subjects are offered VAXCHORA

[a] All subjects: issued at Day 1, reviewed at Day 11

[b] Only if between Day 1 and Day 11 (All subjects)

[c] Telephone contact required; in-person visit as clinically indicated

[d] for females of child-bearing potential

181[PC]: Placebo crossover Vaccination (Day 1)

191[PC]: Placebo crossover 10 days after Vaccination

209[PC]: Placebo crossover 28 days after Vaccination

271[PC]: Placebo crossover 90 days after Vaccination

365[PC]: Placebo crossover 180 days after Vaccination

COHORT 3 2 to <6 years	Study Day:	Screening	1	11	29	91 Phone FU	181	Early D/C	181[PC]	191[PC]	209[PC]	271[PC] Phone FU	365[PC] Phone FU
Window (days):		-30	0	0	±2	±7	±7	n/a	±7	0	±2	±7	±7
Informed Consent/Assent		X[1]											
Medical History		X[1]	X[2]										
Inclusion/Exclusion		X[1]	X										
Physical Exam		X[1]	X[2]										
Vital Signs		X[1]	X						X[PC]				
Con Med Evaluation		X[1]	X[2]	X	X	X[c]	X	X	X[PC]	X[PC]	X[PC]	X[PC]	X[PC]
Vaccination			**X**						X[PC]				
Acceptability/Palatability			X						X[PC]				
Memory Aid [a]			X	X				X[b]	X[PC]	X[PC]			
Solicited AEs			X					X[b]	X[PC]				
Adverse Event Evaluation			X	X	X	X[c]	X	X	X[PC]	X[PC]	X[PC]	X[PC]	X[PC]
SVA			X	X	X			X					

X = All Subjects; PC = Placebo crossovers only; D/C = discontinuation; SVA = serum vibriocidal antibodies

[1] May be conducted at Day 1

[2] Updated as needed

[PC] placebo crossover subjects are offered VAXCHORA

[a] All subjects: issued at Day 1, reviewed at Day 11

[b] Only if between Day 1 and Day 11 (All subjects)

[c] Telephone contact required; in-person visit as clinically indicated

[d] for females of child-bearing potential

181[PC]: Placebo crossover Vaccination (Day 1)

191[PC]: Placebo crossover 10 days after Vaccination

209[PC]: Placebo crossover 28 days after Vaccination

271[PC]: Placebo crossover 90 days after Vaccination

365[PC]: Placebo crossover 180 days after Vaccination

APPENDIX B: TOXICITY GRADING SCALE

Table for Clinical Abnormalities

VACCINE REACTION	MILD (Grade 1)	MODERATE (Grade 2)	SEVERE (Grade 3)	POTENTIALLY LIFE THREATENING (Grade 4)
Abdominal Pain	Mild, no interference with activity	Some interference with activity	Significant, prevents daily activity	ER visit or hospitalization for hypotensive shock
Anorexia (Lack of appetite)	No interference with activity	Some interference with activity	Significant, prevents daily activity	ER visit or hospitalization
Diarrhea	4 loose stools /24 hours	5 loose stools/24 hours	≥ 6 loose stools/24 hours	ER visit or hospitalization
Fatigue (Tiredness)	Mild, no interference with activity	Some interference with activity	Significant, prevents daily activity	ER visit or hospitalization
Fever	> 100.4 – 101.1°F (≥ 38.0 –38.4°C)	≥101.2 – 102°F (≥ 38.5 –38.9°C)	≥ 102.1°F–104°F (≥ 39°C–40°C)	> 104°F (> 40°C)
Headache	Mild, no interference with activity	Some interference with activity	Significant, prevents daily activity	ER visit or hospitalization
Myalgia	Mild, no interference with activity	Some interference with activity	Significant, prevents daily activity	ER visit or hospitalization
Nausea	Mild, no interference with activity	Some interference with activity	Significant, prevents daily activity	ER visit or hospitalization for hypotensive shock
Vomiting	1–2 episodes/24 hours	> 2 episodes/24 hours	Requires IV hydration	ER visit or hospitalization for hypotensive shock "

When developing this Toxicity Grading Scale, PaxVax referred to the recommendations in the FDA's Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Subjects Enrolled in Preventive Vaccine Clinical Trials (US FDA 2007).
Diarrhea 1-3 Stools/24 hours: Record as "loose stools" on the AE CRF.

REFERENCES

Agresti A, Coull BA. (1998). Approximate is better than "exact" for interval estimation of binomial proportions. The American Statistician, 52(2), 119-126.

Ali M, Nelson A, Lopez AL, Sack DA. (2015) Updated Global Burden of Cholera in Endemic Countries. PloS Negl Trop Dis. 2015 Jun 9(6): e0003832.

Cooper PJ, Chico ME, Losonsky G, Sandoval C, Espinel I, Sridhara R, Aguilar M, Guevara A, Guderian RH, Levine MM, Griffin GE, Nutman TB: Albendazole Treatment of Children with Ascariasis Enhances the Vibriocidal Antibody Response to the Live Attenuated Oral Cholera Vaccine CVD 103-HgR. J Infect Dis 2000, 182:1199-1206.

Czerkinsky C, Holmgren J. Vaccines against enteric infections for the developing world. Philos Trans R Soc Lond B Biol Sci.2015 Jun 19; 370(1671): 20150142.

Dziejman M, Serruto D, Tam VC, Sturtevant D, Diraphat P, Faruque SM, et al. Genomic characterization of non-O1, non-O139 *Vibrio cholerae* reveals genes for a type III secretion system. Proc Natl Acad Sci U S A. 2005;Mar 1;102(9):3465–70.

Faucher, J. F., Binder, R., Missinou, M. A., Matsiegui, P. B., Gruss, H., Neubauer, R., ... & Kremsner, P. G. (2002). Efficacy of atovaquone/proguanil for malaria prophylaxis in children and its effect on the immunogenicity of live oral typhoid and cholera vaccines. Clinical infectious diseases, 35(10), 1147-1154.

Formal SB, Kundel D, Schneider H, Kunev N, Sprinz H. Studies with *Vibrio cholerae* in the ligated loop of the rabbit intestine. Br J Exp Pathol. 1961 Oct;42:504–10.

Lagos R, Avendaño R, Prado V, Horwitz I, Wasserman S, Losonsky G, Cryz S Jr, Kaper JB, Levine MM. Attenuated live cholera vaccine strain CVD 103-HgR elicits significantly higher serum vibriocidal antibody titres in persons of blood group 0. Infect Immun 1995 63:707-709.

Lagos R, Losonsky G, Abrego P, San Martin O, Prado V, Wasserman S, Levine MM: Tolerancia, immunogenicidad, excresión y transmisión de la vacuna anti-colera oral viva-atenuada, CVD 103-HgR, estudio pareado de doble ciego en niños Chilenos de 24 a 59 meses. Bol Hosp Infant Mex 1996, 53:214-220.

Lagos R, San Martin O, Wasserman SS, Prado V, Losonsky GA, Bustamante C, Levine MM. Palatability, reactogenicity and immunogenicity of engineered live oral cholera vaccine CVD 103-HgR in Chilean infants and toddlers. Pediatr Infect Dis J. 1999a Jul;18(7):624-30.

Lagos R, Fasano A, Wasserman SS, Prado V, Martin OS, Abrego P, Losonsky GA, Alegria S, Levine MM. Effect of Small Bowl Bacterial Overgrowth on the Immunogenicity of Single-Dose Live Oral Cholera Vaccine CVD 103-HgR. The Journal of Infectious Diseases 1999b;180:1709–12

Levine MM. Immunogenicity and efficacy of oral vaccines in developing countries: lessons from a live cholera vaccine. BMC Biol. 2010 Oct 4;8:129. doi: 10.1186/1741-7007-8-129.

Morris JG Jr, Takeda T, Tall BD, Losonsky GA, Bhattacharya SK, Forrest BD, Kay BA, et al. Experimental non-O group 1 *Vibrio cholerae* gastroenteritis in humans. J Clin Invest. 1990;85(3):697-705.

Orochol [package insert] Swiss Serum and Vaccine Institute Berne (SWITZERLAND)/ CSL Ltd. (AUSTRALIA) 2000

Pierce NF, Cray WC Jr, Kaper JB, Mekalanos JJ. Determinants of immunogenicity and mechanisms of protection by virulent and mutant *Vibrio cholerae* O1 in rabbits. Infect Immun. 1988 Jan;56(1):142–8.

Richardson SH. In: Wachsmuth IK, Blake PA, Olsvik O, eds. Chapter 14. *Vibrio cholerae* and Cholera: Molecular to Global Perspective. 1994. Washington, DC: American Society for Microbiology.

Richie, E., Punjabi, N. H., Sidharta, Y., Peetosutan, K., Sukandar, M., Wasserman, S. S., ... & O'Hanley, P. (2000). Efficacy trial of single-dose live oral cholera vaccine CVD 103-HgR in North Jakarta, Indonesia, a cholera-endemic area. Vaccine, 18(22), 2399-2410.

Russell RG, Tall BD, Morris JG Jr. Non-O1 *Vibrio cholerae* intestinal pathology and invasion in the removable intestinal tie adult rabbit diarrhea model. Infect Immun. 1992 Feb; 60(2):435–42.

Sack D, Qadri F, Svennerholm A-M. Determinants of responses to oral vaccines in developing countries. Ann Nestlé (Engl) 2008;66:71–9.

Simanjuntak CH, O'Hanley P, Punjabi NH, Noriega F, Pazzaglia G, Dykstra P, Kay B, Suharyono, Budiarso A, Rifai AR, et al. Safety, immunogenicity, and transmissibility of single-dose live oral cholera vaccine strain CVD 103-HgR in 24- to 59-month-old Indonesian children. J Infect Dis. 1993 Nov; 168(5):1169-76.

Suharyono, Simanjuntak C, Witham N, Punjabi N, Heppner DG, Losonsky GA, Totosudirjo H, Rifai AR, Clemens J, Lim YL, Burr D, Wasserman SS, Kaper J, Sorenson K, Cryz S, Levine MM. Safety and immunogenicity of single-dose live oral cholera vaccine CVD 103-HgR in 5-9-year-old Indonesian children. Lancet 1992, 340:689-694.

US Food and Drug Administration. "Toxicity Grading Scale for Healthy Adult and Adolescent Vaccinees Enrolled in Preventative Vaccine Clinical Trials" [FDA Guidance 2007].

VAXCHORA [package insert]. Hamilton, Bermuda: PaxVax Bermuda Ltd.; 2016.

World Health Organization. Weekly epidemiological record. Cholera, 2014. No 40, 2015, 90, 517-544, http://www.who.int/wer

Wiedermann, G., Kollaritsch, H., Jeschko, E., Kundi, M., Herzog, C., & Wegmüller, B. (1998). Adverse events after oral vaccination against cholera with CVD103-HgR. Wiener Klinische Wochenschrift, 110(10), 376-378.