STATISTICAL ANALYSIS PLAN

Study 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
Name of Test Drug:	VAXCHORA (Cholera Vaccine, Live, Oral)
Study Number:	PXVX-VC-200-006 (NCT03220737)
Protocol Version:	3.0
Protocol Date:	15 November 2017
Phase of Study:	Phase 4
Analysis Plan Version:	Final 1.0
Analysis Plan Date:	28 November 2017
Analysis Plan Author:	[redacted]

CONFIDENTIAL AND PROPRIETARY INFORMATION

LIST OF ABBREVIATIONS

AE	Adverse Event
CDER	Center for Drug Evaluation and Research
CI	Confidence Interval
CRF	Case report form
eCRF	Electronic Case report form
EDC	Electronic Data Collection
FR	Federal Register
GMT	Geometric Mean Titer
HLGT	High level group term
HLT	High level term
LLOQ	Lower limit of quantification
LLT	Lowest level term
LPS	Lipopolysaccharide
MedDRA	Medical dictionary for regulatory activities
mITT	Modified Intent-to-Treat
PT	Preferred term
SAE	Serious Adverse Event
SAP	Statistical analysis plan
SOC	System organ class
SMC	Safety Monitoring Committee
SVA	Serum Vibriocidal Antibody
ULOQ	Upper limit of quantification
WHO	World Health Organization

1. INTRODUCTION

VAXCHORA (Cholera Vaccine, Live, Oral) is a vaccine indicated for active immunization against disease caused by *Vibrio cholera* 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.

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 Mutacol. 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.

1.1. Study Objectives

Immunogenicity Objectives	The two primary immunogenicity 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. 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.
Safety Objectives	To 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

1.2. Study Design

Design Configuration and Subject Population	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, an observation period from Day 1 to Day 29, and a follow-up period through Day 181. 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 with 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. Primary Comparator Group for All Age Cohorts for primary objective 1: 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.
Investigational 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. VAXCHORA is reconstituted by adding the contents of the buffer sachet to purified bottled water, stirring, adding the contents of the vaccine sachet, and stirring the final suspension. For Cohorts 1 and 2, the dose volume is 100 mL. For Cohort 3, the dose volume is 50 mL. Placebo is normal (0.9%) saline at the same volume as Vaxchora. Stevia may be added to either Vaxchora or placebo as a sweetener. Subject are instructed to drink the full dose of blinded study vaccine (VAXCHORA or placebo) within 15 minutes of reconstitution. . 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.
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 (see above). VAXCHORA and placebo will be administered orally.
Study Population	At least 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) • Cohort 2: 6 to <12 years (n=150:active treatment group, n=25:placebo crossover group) • Cohort 3: 2 to < 6 years (n=210:active treatment group, n=35:placebo crossover group) 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: a. Any other licensed vaccines b. Antibiotics or chloroquine 16. Received or plans to receive any other investigational agent throughout the main study (Day 181).
Study Procedures	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 will be collected through Day 29 and Day 181 through Day 209 (for Placebo Crossover subjects). Serious adverse events 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 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.
Randomization	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. Users will enroll each subject by assigning and entering a Subject ID # in the Electronic Data Collection (EDC) system. The 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. 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 Day181, where neither the sponsor, the statistical team, study volunteer subjects, nor clinical site personnel (except for the unblinded staff), will know subjects' treatment assignment. Subjects, clinical site personnel, and Investigators will remain blinded until a subject has completed their Day 181 visit at which point the subject is unblinded individually and their active/placebo status will be recorded in the EDC. Once all

	safety and immunogenicity data have been received from the Day 181 visit and the data cleaned, and signed by the PI, the biostatistics group will carry out the primary analysis.
Sites	Up to 30 sites in the US
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 6 months 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.

1.3. Sample Size and Power

Planned Sample Size	595 Subjects

Power Statement	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% inevaluable for immunogenicity (30% inevaluable for cohort 3), and, 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.

2. TYPE OF PLANNED ANALYSIS

The analysis plan describes the methods by which the data from this study will be analyzed. Completion of the Day 730 assessment for the final subject in the long-term follow-up subset will mark the end of EDC data collection for the study. Final laboratory data will be received after the Day 730 samples are collected and analyzed.

The final report for the study will occur after all subjects have completed Day 730. An additional report to be used for a possible regulatory filing may be written that covers the data for cohorts 1 and 2 through their Day 181 visit.

3. GENERAL CONSIDERATIONS FOR DATA ANALYSES

Continuous variables will be summarized in terms of the sample size, mean, standard deviation, median, minimum and maximum. The geometric mean and 95% confidence interval (CI) will be presented as appropriate. Calculation of the geometric mean will be performed by exponentiating the mean of the $\log_{10}$-transformed data to convert to the non-transformed scale. Similarly, the calculation of the corresponding 95% CI will be performed. Categorical variables will be summarized using frequency counts and percentages. In general, data will be summarized by age cohort and treatment group. Unless otherwise noted, the denominator for the percentages will include all subjects in the respective treatment group.

The minimum and maximum values will be presented with the same number of decimal places as the raw data collected. The mean and median will be presented using one additional decimal place. The standard deviation and standard error will also be presented using one additional decimal place.

Percentages will be presented to one decimal place in general. The denominator will be the total size of the sample, N, unless otherwise noted. A percentage of 100% will be reported as 100%. No value of 0% will be reported. A computation of a percent that results in 0% will be left as blank, i.e., a numerator of 0 will be reported, however no percentage will be reported.

3.1. Analysis Populations

Analysis populations define which subjects and records are included in an analysis.

The exclusion of a subject from the analysis populations will occur before the study blind is broken for the subject. A summary of the number and percent of subjects in each analysis population will be provided by treatment group, age cohort, and in total.

Analyses based on all populations will be performed according to the corrected age cohort based on the subject's actual age should randomization to the wrong age cohort occur.

3.1.1. Randomized Population

The randomized population includes all subjects who were randomized into the study. Analyses based on the randomized population will be performed according to the treatment group to which a subject was randomized.

3.1.2. Modified Intent-to-Treat (mITT) Population

The modified intent-to-treat population includes all subjects in the randomized population who have both Day 1 and Day 11 SVA assay results. Analyses based on the mITT

population will be performed according to the treatment group to which a subject was randomized.

3.1.3. Immunogenicity Evaluable Population

The immunogenicity evaluable population will include all subjects in the mITT population who:

- Receive the minimum dose of vaccine correctly (see below)
- Have serum vibriocidal assay (SVA) results at the Day 1 and the Day 11 visit within required window
- Have no exclusionary protocol deviation as determined prior to unblinding

A exclusionary deviation is defined as a protocol deviation that could potentially have a significant impact on the immunogenicity result of the subject. These are more explicitly defined below and will be identified prior to unblinding. These deviations and the judgment regarding their use will be listed and summarized in the final clinical study report.

Exclusionary Protocol Deviations

1. Allowed blood draw window:
   Day 1: must be prior to vaccination
   Day 11: must be in period from Day 8 through Day 16

   Blood draws which fall outside of these windows will be considered exclusionary deviations.

2. Dose of vaccine
   - Must have consumed at least 80% of vaccine volume (80 mL for cohorts 1 and 2 or 40 mL for cohort 3).

   Subjects not meeting this criterion will be considered to have an exclusionary protocol deviation.

3. Concomitant antibiotics or non-study concomitant vaccines
   - Any antibiotic or non-study vaccine given within -14/+11 days of vaccination will be decided as an exclusionary violation on an individual antibiotic-type or vaccine-type determination.

4. Wrong treatment given/no treatment given

5. Inclusion/exclusion criteria violations
   - Age at entry – age < 2 or ≥ 18 years

- Prohibited medications – exclusionary deviations will be considered on a case-by-case basis
- Medical history conditions which could interfere with immunogenicity will be decided on a case-by-case decision.

6. Other
   - Other miscellaneous violations discovered during the conduct of the trial, which may fundamentally affect the assessment of immunogenicity (e.g., immunoglobulin or high dose corticosteroid use), will be decided on a case-by-case basis prior to unblinding.

Analysis for the primary immunogenicity objectives will be based on the immunogenicity evaluable population as well as the mITT population. Analyses for secondary immunogenicity objectives will be carried out in the Immunogenicity evaluable population.

3.1.4. Bridging Population

The bridging population comprises all subjects in the PXVX-VC-200-006 immunogenicity evaluable population along with subjects vaccinated with PXVX0200 in the immunogenicity evaluable population from the adult VAXCHORA lot consistency trial PXVX-VC-200-004.

3.1.5. Long-Term Follow-up Sub-study Population

The long-term follow-up sub-study population includes vaccinated subjects with a Day 1 SVA result in cohort 1 who have agreed to participate in the extended follow-up in the study through Day 730.

3.1.6. Memory B cell Population

The memory B cell population includes vaccinated subjects who have agreed to the memory B cell collection (i.e., they had memory B cell samples collected and have both day 1 and any memory B cell results post-vaccination).

3.1.7. Safety Population

The safety population includes subjects who received any amount of study vaccine or placebo. Analyses based on the safety population will be performed according to the treatment group of the study vaccine received.

3.2. Missing Data and Outliers

Missing Data

A missing datum for a given study visit may be due to the fact that:

1. data were not collected for the visit or were unusable, or

2. a subject permanently discontinued from the study before reaching the assessment.

There are no plans to impute values for missing data points except for imputing missing relationship to study drug for AEs as related.

3.3. Data Handling Conventions and Transformations

By-subject listings will be presented for all randomized subjects sorted by subject ID number, visit date, and time (if applicable). Data collected on log forms, such as AEs, will be presented in chronological order within subject. Subjects will be listed according to the actual treatment received.

Baseline is defined as the Day 1 value. If the Day 1 value is missing, then the last non-missing value prior to Day 1 will be used as the baseline value.

Data that are less than the lower limit of quantification (LLOQ) or above the upper limit of quantification (ULOQ) will be imputed as follows:

- SVA assay results that are reported as less than the LLOQ will be imputed as the LLOQ when calculating seroconversion rates and geometric means. For example, if the LLOQ is 20 and a result is noted as "<20", a titer of 20 will be imputed.

- A value that is one unit above the limit of quantitation will be used for calculation of descriptive statistics if the datum is reported in the form of "> x" (where x is considered the limit of quantitation). Values with decimal points will follow the same logic as above.

The limit of quantitation will be used for calculation of descriptive statistics if the datum is reported in the form of "≤ x" or "≥ x" (where x is considered the limit of quantitation).

3.4. Visit Windows

For determination of Baseline visit and all other visits, an analysis visit will be derived to summarize the data by proper visit window interval.

The following algorithm will be used for the study day determination:

- Day 1 = Day of Vaccination;
- If Date of Assessment/Visit >= Date of Vaccination then Study Day = (Date of Assessment/Visit - Date of Vaccination) + 1;
- If Date of Assessment/Visit < Date of Vaccination then Study Day = (Date of Assessment/Visit - Date of Vaccination).

4. SUBJECT DISPOSITION

4.1. Subject Enrollment

The number and percent of subjects randomized will be summarized by site for each age cohort (including all ages combined), by treatment group and across treatment groups within the age cohort. The denominator for this calculation will be the total number of randomized subjects.

4.2. Disposition of Subjects

A summary of subject disposition will be provided by age cohort, treatment group and overall. This summary will present the number of subjects who completed through Day 181, who discontinued from the study early and the primary reason for discontinuing the study early. Similar summaries will be provided for the placebo crossover subjects who choose to receive Vaxchora and also for subjects included in the long-term follow-up sub-study. No p-values or inferences based upon comparison of disposition in the treatments will be generated.

A data listing of reasons for early study discontinuation for the randomized population will be provided as well as a listing of reasons for screen failure.

5. BASELINE DATA

5.1. Demographics

Subject demographic data (e.g., age, sex, weight, height, body mass index (BMI), race, and ethnicity) will be summarized by age cohort, treatment group and overall using descriptive statistics for continuous data and by the number and percent of subjects for categorical data. Age is calculated in years at the time of vaccination. No p-values or inferences regarding comparisons of baseline demographics between the two treatment groups will be provided.

The summary will be provided for the randomized, mITT, immunogenicity evaluable, bridging, Memory B cell, safety, and long-term follow-up sub-study populations. A listing of demographic data will be provided for the randomized population.

5.2. Medical History

Medical history will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 15.0. Medical history will be summarized by system organ class (SOC), preferred term, treatment group and overall for the randomized population. Medical history will also be listed based on the randomized population.

6. PRIMARY AND SECONDARY ANALYSES

The treatment period begins and ends at the time of vaccination at baseline (Day 1). The observation period spans Day 1 post-vaccination through Day 29, and follow-up period spans the time following the Day 29 visit through Day 181 (or through Day 365 for the Placebo Crossover subjects or 730 for the long-term follow-up sub-study subjects).

The primary and secondary analyses will be performed once all data have been collected from all subjects through their Day 181 (or Day 730, depending on study report purpose) follow-up visit and all EDC records have been cleaned and PI signed, and all laboratory data have been cleaned.

The primary endpoint will be analyzed based on the bridging population and repeated on the mITT population for robustness purposes. The secondary and exploratory endpoints will be based on only the immunogenicity evaluable population (including the immunogenicity sub-study population, as applicable).

6.1. Primary Endpoints

The primary endpoint is:

- Seroconversion Rate at Day 11: 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.

6.1.1. Analysis Methods for Primary Endpoints

For each age cohort, a two-sided 98.3% confidence interval of the Day 11 seroconversion rate will be computed using the Wilson method (Agresti 1998). The lower bound of this interval must equal or exceed 70%. Fisher's exact test will be used to test equality of seroconversion across groups.

The Day 11 seroconversion rate for vaccines in each of the 3 age cohorts will also 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 (the "004 bridging population") by calculating the difference between the two rates and computing a 96.7% confidence interval for this difference based on Newcombe hybrid score method. 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.

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 immunogenicity objective of establishing non-inferiority relative to the 004 bridging population, and 1/3 of the alpha will be allotted to the primary immunogenicity objective of demonstrating that the seroconversion rate equals or exceeds 70%. The two primary immunogenicity objectives will be evaluated independently, and within each objective, testing in the different age cohorts will proceed sequentially beginning with the data for cohort 1, as follows:

- Non-inferiority: Analysis of non-inferiority between Cohort 1 and the 004 bridging population will be conducted first. Analysis of non-inferiority in Cohort 2 will be conducted only if the prespecified acceptance criterion is met for Cohort 1. Analysis of non-inferiority in Cohort 3 will be conducted only if the pre-specified acceptance criteria are met for Cohorts 1 and 2. This strategy maintains the overall Type I error for the primary objective of non-inferiority at alpha = 0.033.

- Seroconversion Rate Lower Limit ($\geq$ 70%) Criterion: the analysis of the seroconversion rate for cohort 1 will be conducted first. Formal analysis of the acceptability of the seroconversion rate in cohort 2 will be performed only if the lower bound of the two-sided 98.3% confidence interval on the seroconversion rate in cohort 1 meets the pre-specified acceptance criterion. Formal evaluation of the acceptability of the seroconversion rate in cohort 3 will occur only if the acceptance criteria are met for both cohorts 1 and 2. This strategy maintains the overall Type I error for the primary objective concerning the minimum seroconversion rate at alpha =0.017.

As an additional sensitivity analysis, a logistic regression will be performed for seroconversion. Significance of the comparison between the bridging population groups will be assessed via the type III test of the age group effect from a logistic regression of seroconversion at Day 11 with age group, baseline titer, and gender as predictors in the model. The estimate of the adjusted difference between the groups and its confidence interval will be also be derived from this logistic model.

6.2. Secondary Endpoints

Secondary endpoints include:

- Seroconversion of SVA against *V. cholerae* at Day 29 for all subjects.
- Seroconversion of SVA against *V. cholerae* at Days 91and 181 for subjects in cohort 1.
- Seroconversion of SVA against *V. cholerae* at Days 365, 547 and 730 for subjects participating in the sub-study.

6.2.1. Analysis Methods for Secondary Endpoints

The seroconversion rate, cumulative seroconversion rate, and 95% confidence intervals at Day 29 will be estimated for each treatment group for each cohort and across all cohorts. For cohort 1, the seroconversion rates, cumulative seroconversion rates, and 95% confidence intervals at Days 91 and 181 will be estimated for each treatment group. For cohort 1 subjects participating in the sub-study, the seroconversion rates, cumulative seroconversion rates, and 95% confidence intervals at Days 365, 547 and 730 will be estimated. The 95% CIs will calculated using the Wilson method (Agresti 1998).

To provide a global rate of response over all age cohorts in the study (ages 2-<18), a summary of Vaxchora vs. Placebo subjects will also be provided for each of the collected visits where sera was collected.

The cumulative seroconversion rate is defined as the cumulative number of subjects who meet the seroconversion criterion at or prior to that visit. The denominator for the rate is the same at each time point and is equal to the number of subjects in the treatment group who had a baseline assay result and at least one post-vaccination assay result.

SVA titers will also be summarized by Geometric Mean Titer (GMT) at each visit where collected including Day 1. To calculate the GMT and 95% CI, the SVA results will be $\log_{10}$-transformed, the mean and 95% CI of these transformed data will be calculated and then exponentiated to convert to the non-transformed scale. Any SVA result reported less than the LLOQ will be handled according to Section 3.3 prior to $\log_{10}$-transformation. Geometric mean point estimates, together with their 95% CIs, and the p-value are based on t-statistics assuming normal distribution of the log titer. Median point estimates and their 95% CIs will be distribution-free estimates.

A baseline value will be defined as the last available value collected prior to the vaccination. The geometric mean and median fold-increase in titer over baseline will also be presented at each visit. To calculate the geometric mean fold-increase and 95% CI, the fold-increase results will be $\log_{10}$-transformed, the mean and 95% CI of these transformed data will be calculated

and then exponentiated to convert to the non-transformed scale. A t-test will be used to compare the log_{10}-transformed fold increase between PXVX0200 and placebo subjects.

6.3. Exploratory Endpoints

Exploratory endpoints include:

- Acceptability endpoint: The percent of subjects in each cohort who completed dosing according to protocol. This is defined as the entire volume of dose being consumed within 15 minutes after reconstitution.

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

Other exploratory endpoint:

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

6.3.1. Analysis Methods for Exploratory Endpoints

The number and percent of subjects in each cohort who completed dosing according to protocol by treatment group will be summarized for the randomized population. Acceptability by palatability score will also be presented.

Subject and caregiver responses to the 5-point Hedonic scale will be summarized descriptively by cohort and treatment group for the randomized population.

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 mean, 95% CI, median, minimum and maximum percentages, as well as median and geometric mean fold rise from Day 1 to Day 91, Days 181, 365, 547, 730 and also Day 91 to subsequent timepoints, will be presented for the memory B cell population. Median point estimates and their 95% CIs will be distribution-free estimates. The Wilcoxon signed-rank test will be used to assess the within-subject difference between results (log[fold rise]) from two different time points.

As a purely descriptive summary, an additional exploratory analysis included will be a summary of the number and percent of subjects in each group at each timepoint whose SVA titer is less than the LLOQ.

7. SAFETY ANALYSES

7.1. Adverse Events

An AE is any untoward medical occurrence in a study participant, regardless of the suspected causal relationship with study vaccine. An AE can therefore be a new-onset symptom or disease, an exacerbation of a pre-existing symptom or disease, 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 reporting period for SAEs begins at the time of informed consent and continues for the duration of study participation. 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 study vaccine administration on Day 181 and continues through Day 188. The reporting period for all other unsolicited AEs begins after study vaccine or placebo administration on Day 1 and continues through Day 29.

An additional reporting period for unsolicited AEs for the Placebo Crossover who choose to receive PXVX0200 begins after study vaccine administration on Day 181 and continues through Day 209.

Solicited AEs are characterized by the following pre-specified signs and symptoms: abdominal pain, headache, lack of appetite, tiredness, diarrhea, nausea, vomiting, and fever. Solicited adverse events from Day 1 through Day 8 will be recorded on the solicited adverse event eCRF. Symptoms experienced on Day 8 and continuing beyond Day 8 will be recorded on the adverse event eCRF with a start date corresponding to the first date the symptom was continuously experienced through Day 8. Analogous methods will be used to document solicited AEs in the subjects who receive vaccine at Day 181 in the Placebo Crossover group.

Unsolicited AEs are defined as an AE that is spontaneously reported by the subject or discovered by the Investigator.

Adverse Event Dictionary

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) version 15.0. System Organ Class (SOC), High Level Group Term (HLGT), High Level Term (HLT), Preferred Term (PT), and Lowest Level Term (LLT) will be attached to the clinical database.

With the exception of diarrhea, the preferred term (PT) will be used in the summary tables. If the PT is "diarrhoea", the event used in the summaries will be the LLT, rather than the PT, in order to separate loose stools (fewer than 4 per day) from mild diarrhea (4 or more loose stools per day), as defined in the protocol toxicity grade scale. This approach is being used for consistency with previous VAXCHORA studies, including PXVX-VC-200-004.

7.1.1. Adverse Event Severity

AEs (inclusive of all solicited and unsolicited adverse events) are graded by the Investigator or designee as Grade 1 (mild), Grade 2 (moderate), Grade 3 (severe) or Grade 4 (potentially life-threatening) according to toxicity criteria specified in the study protocol (see Appendix B of study protocol). The severity grade of events for which the Investigator did not record severity will be categorized as "missing" for tabular summaries and data listings, and will be considered the least severe for the purposes of sorting for data presentation (will sort to the top).

7.1.2. Relationship of Adverse Events to Study Drug

AEs and SAEs are determined to be related or unrelated to study product by study Investigators or their designees. The Investigators or designees will evaluate the relatedness of an AE to vaccine treatment using three categories: Not Related, Possibly Related, and Probably Related. Related AEs are those for which the Investigator or designee answers "Possibly Related" or "Probably Related". Events for which the Investigator or designee did not record relationship to study drug will be considered related to study drug for the purposes of analysis. Data listings will show relationship as missing in this case.

7.1.3. Serious Adverse Events

Serious adverse events (SAEs) are those identified in the clinical database as serious by the Principal Investigator or designee. Further information on the definition of a SAE is provided in Section 7.1.4 of the study protocol.

7.1.4. Summaries of Adverse Events and Deaths

A single summary table will capture all the high-level findings of AE summaries by tabulating, by treatment group and age cohort, the number and percentage of subjects who (1) had any solicited or unsolicited AE, (2) had any solicited AE, (3) had any unsolicited AE, (4) had any treatment-related solicited or unsolicited AE, (5) had any solicited treatment-related AE, (6) had any unsolicited treatment-related AE, (7) had any SAE, (8) had any treatment-related SAE (9) permanently discontinued from study due to a solicited or unsolicited AE, and (10) death during study.

Summaries (number and percent of subjects) of AEs will be provided by SOC and PT and cohort and treatment group as follows:

- All AEs (inclusive of all solicited and unsolicited AEs)
- All unsolicited AEs
- Treatment-related unsolicited AEs
- AEs that caused permanent discontinuation from study,
- Unsolicited AEs by maximum severity grade,
- Treatment-related unsolicited AEs by maximum severity grade.

For subjects with multiple events, only one event will be counted in each summary. For data presentation, SOCs will be ordered alphabetically, with PT sorted by decreasing total frequency. Solicited AEs will be presented by decreasing total frequency. For summaries by maximum severity grade, only the event with the highest severity will be presented. For summaries by relatedness, only one event per relatedness category will be presented. Summaries will be provided by treatment group and for the Safety Analysis Set.

In addition to the by-treatment summaries, data listings will be provided for the following:

- All unsolicited AEs
- All solicited events
- SAEs
- Deaths

7.2. Safety Endpoints

Safety endpoints include:

- Incidence and severity of solicited events through Day 8
    - Solicited adverse events include: abdominal pain, headache, lack of appetite, tiredness, diarrhea, nausea, vomiting and fever.
- Incidence and severity of unsolicited adverse events through Day 29.
- Incidence of serious adverse events (SAE) through Day 181

7.2.1. Analysis Methods for Safety Endpoints

The number and percentage of subjects who experience a solicited AE recorded during Days 1 through 8 will be summarized according to maximum severity grade by age group, treatment group and decreasing total frequency. For subjects with multiple events of the same type, only the event of the highest severity will be counted in each summary. Events with missing severity grades will be handled according to Section 7.1.1. Fisher's exact test will be used to compare the frequency of the corresponding solicited event between PXVX0200 and placebo. This summary will be repeated for treatment-related solicited AEs and solicited AEs during Days 1 through 8 of at least severe (Grade 3) severity or higher.

Day of onset will be summarized with descriptive statistics by age group and treatment group. The median and its 95% CI will be estimated by Kaplan-Meier analysis. The log-rank test will be used to compare day of onset between PXVX0200 and placebo. This summary will be repeated for treatment-related solicited AEs. Also included will be a summary of the number and percentage of subjects reporting each solicited event on each day of the Day 1 to 8 reporting period.

The number of days a subject experiences each solicited AE will be summarized by descriptive statistics (number of subjects with each solicited AE, mean, standard deviation, median, minimum and maximum) by age group and treatment group. The mean and its 95% CIs will be based on t-statistics assuming normal distribution. The median and its 95% CIs will be distribution free estimates. A Wilcoxon rank sum test on the number of days of symptoms will be used to compare PXVX0200 and placebo. This summary will be repeated for treatment-related solicited AEs.

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.

A summary of the number and percentage of subjects who experience an unsolicited AE during Days 1 through 29 by SOC and PT will be presented. Similarly, a summary of the number and percentage of subjects who experience a treatment-related AE during Days 1 through 29 by SOC and PT will be provided. Non-serious unsolicited AEs with onset date after Day 29 will be summarized with Days 1 through 29. Unsolicited AEs collected from Day 181 through Day 209 will be summarized separately for the Placebo Crossover subjects.

In addition, a summary of the number and percentage of subjects who experience an SAE during Days 1 through 181 (Day 730 for subjects in the sub-study) by SOC and PT will be presented. Similarly, a summary of the number and percentage of subjects who experience a

treatment-related SAE during Days 1 through 181 (or Day 730 for sub-study subjects) by SOC and PT will be provided.

7.3. Prior and Concomitant Medications

Only medications taken from 30 days prior to vaccination through study termination will be recorded.

Any medications started and stopped prior to or on the date of vaccination will be considered prior medications. If a partial stop date is entered and the month and year (if day is missing) or year (if day and month are missing) of the stop date are before the date of vaccination, the medication will be considered a prior medication.

Any medications started prior to or on date of vaccination and continued to be taken after date of vaccination, or started after the date of vaccination will be considered a concomitant medication. If a partial stop date is entered and the month and year (if day is missing) or year (if day and month are missing) of the stop date are after the date of vaccination, the medication will be considered a concomitant medication.

Concomitant medications (i.e., medications other than study vaccine that are taken while receiving study vaccine) and prior medications (medications started and ended before receiving study vaccine) will be coded using the World Health Organization (WHO) Drug Dictionary version March 2017. The WHO preferred name and drug code will be attached to the clinical database. All concomitant medications associated with unsolicited AEs will be documented through Day 29. Concomitant medications associated with SAEs will be documented through Day 181 (and Day 730 for sub-study subjects).

Use of concomitant and prior medications up to and including Day 29 will be summarized (number and percentage of subjects) by treatment group, WHO drug class, and WHO generic name. Multiple drug use (by preferred name) will be counted once only per subject. The summary will be sorted alphabetically by drug class and then by decreasing total frequency within a class. Concomitant medications reported with start dates after Day 29 through Day 181 for all subjects, and through Day 365 for Placebo Crossover subjects who are vaccinated at Day 181, and additionally through Day 730 for Cohort 1 sub-study subjects, will be listed.

Summaries of prior and concomitant medications will be provided for the safety population. No p-values or inferences regarding comparisons of the usage of concomitant medications in the two treatment groups will be generated.

A listing of prior and concomitant medications will be provided, with a column indicating if a medication is prior, Y or N.

7.4. Vital Signs

Vital signs will be listed by subject and timepoint for the randomized population.

7.5. Physical Examination

A data listing will be provided for physical examination results based on the randomized population.

7.6. Other Safety Measures

A data listing of all pregnancy test results will be provided for subjects.

8. REFERENCES

[1] U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Guideline for Industry: E3 Structure and Content of Clinical Study Reports *Federal Register*. July 17, 1996 (61 FR 37320).

[2] U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER). Guidance for Industry: E9 Statistical Principles for Clinical Trials. *Federal Register*. September 16, 1998 (63 FR 49583).

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

9. SOFTWARE

SAS Software Version 9.4. SAS Institute Inc., Cary, NC, USA.

10. SAP REVISION

Revision Date (dd month, yyyy)	Section	Summary of Revision	Reason for Revision