STATISTICAL ANALYSIS PLAN
for
PATH Protocol CVIA 064

Study Title:

A Phase 1 Double-blind, Placebo-controlled, Dose Escalating Study of Intramuscular Detoxified *Shigella flexneri* 2a Artificial Invasin Complex (Invaplex$_{AR\text{-}DETOX}$) Vaccine

Version 2.0

Date: 18Aug2020

Protocol Title	A Phase 1 Double-blind, Placebo-controlled, Dose Escalating Study of Intramuscular Detoxified *Shigella flexneri* 2a Artificial Invasin Complex (Invaplex$_{AR-DETOX}$) Vaccine.
Protocol Number:	CVIA 064, Version 6.0
Development Phase:	Phase 1
Products:	Detoxified *Shigella flexneri* 2a Artificial Invaplex (Invaplex$_{AR-DETOX}$) (Lot 1972). Normal saline.
Form/Route:	Intramuscular
Indication Studied:	*Shigella* prevention
Sponsor:	PATH Vaccine Solutions (PVS) 455 Massachusetts Ave., NW, Suite 1000, Washington, DC 20001
Date of this Plan:	18 Aug 2020
Version Number:	2.0

This study was performed in compliance with Good Clinical Practice.

CVIA 064 STATISTICAL ANALYSIS PLAN REVISION HISTORY

Version Number	Version Date	Summary of Changes
0.1	15 JUL 2019	Original text draft
0.2	04 OCT 2019	Emmes response to PATH and NMRC input
0.3	07 NOV 2019	Emmes response to PATH and NMRC input
0.4	20 NOV 2019	Update to Table 12
1.0	27 NOV 2019	Clean Final
2.0	18 AUG 2020	Table 8 edit Day 57 to 2 weeks post-vac. 3 (not 3 weeks) Table 13 and Table 33 edit for correct dose levels Table 20 and Table 22 remove SOC from table title Mark tables as "In text" as applicable for the CSR. Created immunology summary table shells A1-A4

SIGNATURE PAGE

PROTOCOL NUMBER: CVIA 064: v6.0

STATISTICAL ANALYSIS PLAN: v2.0

PATH Statistician

Signed: Laina Mercer 08/19/2020 11:54 AM EDT

I am approving this document.

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LIST OF ABBREVIATIONS

Abbreviation	Explanation
µg	Microgram(s)
AE	Adverse event, adverse experience
ALS	Antibody lymphocyte supernatant
ASC	Antibody-secreting cell
C	Celsius
cGMP	Current good manufacturing practice(s)
CI	Confidence interval
CSR	Clinical Study Report
CTC	Clinical Trials Center
eCRF	Electronic case report form
F	Fahrenheit
FDA	US Food and Drug Administration
GCP	Good clinical practice(s)
GMI	Geometric Mean (Fold) Increase
GMT	Geometric Mean Titer
HBsAG	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV	Human immunodeficiency virus
HRPO	Human Research Protections Office
ICH	International Council on Harmonisation
IgA	Immunoglobulin A
fIgA	Fecal immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IM	Intramuscular(ly)
IND	Investigational New Drug
$Invaplex_{AR}$	Artificial Invaplex
$Invaplex_{AR\text{-}DETOX}$	Detoxified Artificial Invaplex
IRB	Institutional Review Board
LPS	Lipopolysaccharide antigen
LLOQ	Lower Limit of Quantification
mL	Milliliter(s)
MOP	Manual of Procedures

List of Abbreviations (*continued*)

NMRC	Naval Medical Research Center
PBMC	Peripheral Blood Mononuclear Cells
PI	Principal investigator
PSRT	Protocol Safety Review Team
PT	Preferred Term
PVS	PATH Vaccine Solutions
RCD curve	Reverse cumulative distribution curve
S. flexneri	*Shigella flexneri*
SAE	Serious adverse event
SAP	Statistical analysis plan
SBA	Serum bactericidal activity
SDCC	Statistical and Data Coordinating Center: Emmes
SOC	System Organ Class
ULOQ	Upper Limit of Quantification
WHO	World Health Organization
WRAIR	Walter Reed Army Institute of Research

1. PREFACE

This Statistical Analysis Plan (SAP) for "A Phase 1 Double-blind, Placebo-controlled, Dose Escalating Study of Intramuscular Detoxified *Shigella flexneri* 2a Artificial Invasin Complex (Invaplex$_{AR-DETOX}$) Vaccine" (PATH protocol CVIA 064) describes and expands upon the statistical information presented in the protocol.

This document describes all planned analyses and provides reasons and justifications for these analyses. It also includes sample tables, figures, and listings planned for the final analyses (see **Appendix A**, **Appendix B**, and **Appendix C**). Regarding the final analyses and Clinical Study Report (CSR), this SAP follows the International Conference on Harmonisation (ICH) of Technical Requirements for Registration of Pharmaceuticals for Human Use Guidelines, as indicated in Topic E3 (Structure and Content of Clinical Study Reports), and more generally is consistent with Topic E8 (General Considerations for Clinical Trials) and Topic E9 (Statistical Principles for Clinical Trials). The structure and content of the SAP provide sufficient detail to meet the requirements identified by the US Food and Drug Administration (FDA) and ICH, while all work planned and reported for this SAP will follow internationally accepted guidelines published by the American Statistical Association and the Royal Statistical Society for statistical practice.

This document contains a review of the study design, general statistical considerations, comprehensive statistical analysis methods for safety and immunogenicity outcomes, and a list of proposed tables and figures. Any deviation from this SAP will be described and justified in protocol amendments and/or in the CSR, as appropriate. The reader of this SAP is encouraged to also review the study protocol for details on conduct of the study and the operational aspects of clinical assessments.

2. INTRODUCTION

The main purpose of this study is to assess the safety and tolerability of three doses of Invaplex$_{AR-DETOX}$ in healthy adults. Immunologic responses as determined by IgA and IgG antibodies against Invaplex in Lymphocyte Supernatant (ALS) and serum samples will also be evaluated. Secondary immunologic evaluations (an exploratory objective) may include serologic and ALS responses to additional antigens (*S. flexneri* 2a LPS, IpaB, IpaC), IgG subclasses, IgM responses, and serum bactericidal antibody (SBA) titers against *S. flexneri* 2a 2457T. Stool and saliva samples will be collected to assess antigen-specific fecal IgA responses.

Up to sixty subjects 18-50 years of age will be enrolled into one of three different dose groups. All subjects will be randomized to receive three doses three weeks apart of either study vaccine or placebo, via intramuscular (IM) injection. The study will be initiated with the lowest dose level (2.5 μg) and will proceed to the next highest dose (10 μg) and then the highest dose (25 μg) in an escalating fashion. A dose-level with no occurrence of stopping criteria in the 7 days following the third vaccine dose will prompt moving to the next dose level. All safety data will be summarized and reviewed by a blinded Protocol Safety Review Team (PSRT) prior to dose-escalation.

This Statistical Analysis Plan describes the statistical methodology and summaries required to assess the demographics, safety, reactogenicity and immunogenicity of three doses of detoxified *Shigella flexneri* 2a Invaplex$_{AR}$ (Invaplex$_{AR-DETOX}$) vaccine when administered to healthy adult subjects.

3. STUDY OBJECTIVES AND ENDPOINTS

Study objectives and endpoints from the protocol are described below, with added detail.

3.1. Study Objectives

3.1.1. Primary Objective

The primary objective of the study is to evaluate the safety of Invaplex$_{AR\text{-}DETOX}$ administered by IM immunization. This will include an assessment of the tolerability and safety of Invaplex$_{AR\text{-}DETOX}$ (or placebo) after each dose in terms of rates of solicited local and systemic adverse events (AEs) through 7 days post-vaccination (i.e., on the day of vaccination and 7 subsequent days), unsolicited AEs through 28 days post-final vaccination, hematology, serology, and chemistry abnormalities 7 days post-vaccination, and serious health problems or hospitalizations through approximately 6 months after final study vaccination.

3.1.2. Secondary Objectives

The secondary objective is to evaluate serum IgG/IgA and ALS IgG/IgA following IM immunization with Invaplex$_{AR\text{-}DETOX}$. Baseline serum samples obtained on day 1, prior to vaccination will be used to compare anti-Invaplex endpoint titers to those collected on days 22, 43, 50, 57 and 71. Similarly, anti-Invaplex antibodies from lymphocyte supernatant collected at baseline and on days 8, 29, 50 and 71 will be assessed.

3.1.3. Exploratory Objectives

The exploratory objectives were as follows:

- Collect samples from vaccinated subjects for exploratory immunological assays.
- Perform secondary immunology analyses.
- Collect samples for evaluation of C-reactive protein (CRP) responses to the vaccine and placebo.

3.2. Study Endpoints

3.2.1. Primary Endpoints

3.2.1.1. Reactogenicity and Safety

Solicited local and general AEs through 7 days after each vaccination:

- Occurrence and severity of solicited local AEs including site pain, tenderness, swelling, induration, redness and pruritus in each study group.
- Occurrence and severity of solicited systemic AEs including fever, nausea, vomiting, abdominal pain, diarrhea (loose stools), appetite change, fatigue, headache, myalgias, arthralgias and malaise in each study group.

Unsolicited AEs post-vaccination:

- Occurrence and severity of unsolicited AEs from day 1 to day 71.
- Clinical laboratory abnormalities 7 days after each vaccination: Occurrence of hematological (red blood cells, white blood cells, differentials, hematocrit, platelet count and hemoglobin) or biochemical (sodium, potassium, glucose, AST, ALT, BUN and creatinine) laboratory-based adverse events in each study group.

Occurrence of serious adverse events (SAEs) at any time during the study.

3.2.2. Secondary Endpoints

IgG and IgA serologic and ALS responses to *S. flexneri* 2a Invaplex:

The following aggregate variables will be calculated for the above parameters with 95% confidence interval (CI):

- Serum IgA and IgG geometric mean titers (GMTs) on days 1, 22, 43, 50, 57 and 71.
- ALS IgA and IgG GMTs at baseline and on days 8, 29, 50 and 71.
- Percentage of subjects with a ≥ 4-fold increase in serum IgA and IgG from baseline (day 1) to day 22 (21 days post-vaccination 1), day 43 (21 days post-vaccination 2), 7, 14 and 28 days post-third vaccination, and from baseline to any post-vaccination time.
- Percentage of subjects with a ≥ 4-fold increase in ALS IgA and IgG from baseline to day 7 post each vaccination, day 28 post-third vaccination and from baseline to any post-vaccination time.
- Geometric mean fold-rise (GMFR) in serum IgA and IgG from baseline (day 1) to day 22 (21 days post-vaccination 1), day 43 (21 days post-vaccination 2), days 7, 14 and 28 post-third vaccination, and from baseline to any post-vaccination time.
- Geometric mean fold-rise (GMFR) in ALS IgA and IgG from baseline to day 7 post each vaccination and day 28 post-third vaccination.

3.2.3. Exploratory Endpoints

Exploratory (secondary) immunogenicity endpoints may include serologic and ALS responses to additional antigens (*S. flexneri* 2a LPS, IpaB, IpaC), additional IgG and IgM subclass responses, and serum bactericidal antibody (SBA) titers against *S. flexneri* 2a 2457T. Stool samples will also be collected to assess antigen-specific fecal IgA responses.

3.3. Study Definitions and Derived Variables

3.3.1. Adverse Event (AE)

An AE, as defined by the ICH guideline for GCP, is "Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related."

An AE is considered any adverse change or exacerbation from a baseline condition that occurs following the initial administration of an investigational product whether or not the event is considered to be related to the investigational product. Examples of this include but are not limited to the following:

- Adverse changes including new signs and symptoms, intercurrent illness modifying the clinical course, or the worsening of a baseline condition including the increased frequency of an event or an increased intensity of a condition
- Concomitant disease with onset or increased severity after the start of study product administration
- A new pattern in a preexisting condition occurring after the receipt of investigational product that may signal a clinically meaningful change
- Clinically significant changes in laboratory values

This definition includes exacerbations of pre-existing conditions. Stable pre-existing conditions which do not change in nature or severity during the study are not considered AEs; however, these should be reported as part of the medical history. However, if this condition deteriorates (e.g., increases in frequency or severity grade) during the study, it should be recorded as an AE.

3.3.1.1. Solicited Local and Systemic Reactions

Solicited AEs are pre-specified local and general (systemic) adverse events that are common or known to be associated with vaccinations or the study product. They are actively monitored as indicators of vaccine reactogenicity. Solicited adverse events with onset after the solicitation period should be captured as unsolicited AEs.

3.3.1.2. Unsolicited Adverse Events

Unsolicited AEs are any AEs reported spontaneously by the subject, observed by the study personnel during study visits or identified during review of medical records or source documents, such as diary cards.

3.3.1.3. Suspected Adverse Reactions

An AE, as defined by the ICH guideline for GCP, is "Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related."

Suspected adverse reaction means any AE for which there is a reasonable possibility that the drug caused the AE. For the purposes of IND safety reporting, "reasonable" possibility means there is evidence to suggest a causal relationship between the drug and the adverse event.

Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

An AE is considered any adverse change or exacerbation from a baseline condition that occurs following the initial administration of an investigational product whether or not the event is considered to be related to the investigational product. Examples of this include but are not limited to the following:

- Adverse changes including new signs and symptoms, intercurrent illness modifying the clinical course, or the worsening of a baseline condition including the increased frequency of an event or an increased intensity of a condition
- Concomitant disease with onset or increased severity after the start of study product administration
- A new pattern in a preexisting condition occurring after the receipt of investigational product that may signal a clinically meaningful change
- Clinically significant changes in laboratory values

3.3.2. Serious Adverse Event (SAE) or Serious Suspected Adverse Reaction

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

- Death
- Life-threatening AE
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Congenital anomaly/birth defect (Abortion, stillbirth, and any malformation/disease must be reported as an SAE.)

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

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic reactions or bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

3.3.3. Unexpected Adverse Event or Unexpected Suspected Adverse Reaction

An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed. It can also be considered "unexpected" if an Investigator's Brochure is not required or available and is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the Investigator's Brochure referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the Investigator's Brochure listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to AEs or suspected adverse reactions that are mentioned in the Investigator's Brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug but are not specifically mentioned as occurring with the particular drug under investigation.

3.3.4. Other Adverse Events

Other AEs will be identified by the PI during the evaluation of safety data. Significant AEs of particular clinical importance, other than SAEs and those AEs leading to discontinuation of the subject from the study will be classified as other AEs. For each, a narrative may be written and included in the clinical study report.

3.3.5. Relationship to Investigational Product (Assessment of Causality)

The PI must assign a relationship of each AE to the receipt of the investigational product. The investigator will use clinical judgment in conjunction with the assessment of a plausible biologic mechanism, a temporal relationship between the onset of the event in relation to receipt of the investigational product, and identification of possible alternate etiologies including underlying disease, concurrent illness, or concomitant medications. The following guidelines will be used by investigators to assess the relationship of an AE to study product administration.

Not related: No relationship to the study product. Applies to those events for which evidence exists that there is an alternate etiology.

Unlikely: Likely unrelated to the investigational product. Likely to be related to factors other than the study product but cannot be ruled out with certainty.

Possible: An association between the event and the administration of the investigational product cannot be ruled out. There is a reasonable temporal association, but there may also be an alternative etiology such as the subject's clinical status or underlying factors including other therapy.

Probable: There is a high degree of certainty that a relationship to the investigational product exists. There is a reasonable temporal association, and the event cannot be explained by known characteristics of the subject's clinical state or factors including other therapy.

Definite: An association exists between the receipt of the investigational product and the event. An association to other factors has been ruled out.

3.3.6. Definitions and Derivations Used in This Study

- A baseline value will be defined as the last value obtained prior to the first vaccination of study product.
- Age will be calculated from the date of first vaccination and will be presented in whole years.
- Fever: oral temperature ≥ 38.0°C or 100.4°F.
- For immunology, reciprocal endpoint titers less than the starting dilution (LLOQ) of the assay will be assigned a value of half the starting dilution for computational purposes. For all statistics and listings, values <LLOQ will be presented as "<LLOQ", with LLOQ replaced with the actual starting dilution.

4. INVESTIGATIONAL PLAN

4.1. Overall Study Design and Plan

This is a double-blinded, placebo-controlled, dose-escalating study in which a total of 60 subjects will receive three vaccinations (days 1, 22 and 43) of one of three doses of Invaplex$_{AR\text{-}DETOX}$ (2.5, 10, or 25 µg) or placebo as per **Table 1**. A complete 3-dose series will be completed for an entire cohort at one dose level prior to advancing to the next dose level. Prior to advancing to the next dose level, a blinded safety analysis (including events up to 7 days after the third dose) will be completed and reviewed by the Protocol Safety Review Team (PSRT). If no halting criteria are met, the next cohort and dose level will be initiated.

As an added precaution in the event of severe reactogenicity when Invaplex$_{AR\text{-}DETOX}$ is administered intramuscularly, five subjects of Cohort A will be enrolled in a pilot group prior to enrolling the remaining subjects. In this pilot group, four subjects will receive 2.5 µg of Invaplex$_{AR\text{-}DETOX}$ and one subject will receive placebo (saline). These subjects will be monitored for 7 days post vaccination for solicited and systemic reactions, unsolicited adverse events, and serious adverse events. If no unexpected symptoms occur within 7 days of vaccination, enrollment of the remaining subjects in Cohort A may proceed.

Blood and stool specimens will be collected at prescribed intervals to examine systemic and mucosal immune responses. Vaccine safety will be actively assessed at vaccination and for 28 days following receipt of the third vaccine dose. The decision to advance to the next cohort (higher dose level) will be based on the safety assessment (not immunogenicity). A dose level with no occurrence of stopping criteria will prompt moving to the next cohort. All safety data will be summarized and reviewed by the PSRT prior to dose-escalation

The study consists of 11 visits not including the screening visit or the day -7 visit. Additionally, subjects are expected to be available for a telephone follow-up approximately 6 months after receipt of the last vaccine dose. The clinical protocol time and events schedule is shown in **Table 2**.

4.2. Discussion of Study Design

CVIA 064 is a double-blinded, placebo-controlled, dose-escalating, single center study to assess the safety, reactogenicity and immunogenicity of three doses of Invaplex$_{AR\text{-}DETOX}$ Vaccine when administered to 18 through 50-year-old healthy subjects. As this is a Phase 1 study, the inclusion of placebos in the study design was intended primarily to keep subjects and investigators blinded to the actual product received, rather than as a comparator for safety and immunogenicity outcomes. A total of 60 subjects were planned for this study, the sample size being limited by the early stage of the product concept/testing. The study was designed to evaluate preliminary safety data but not designed to show statistically significant differences between groups.

More detailed information on the study design can be found in the protocol Section 8.

4.3. Selection of Study Population

4.3.1. Description of Study Population

This study will be conducted at the Walter Reed Army Institute of Research (WRAIR) Clinical Trials Center (CTC), Silver Spring, Maryland in the US. The intent is to enroll a total of 60 subjects aged 18 through 50 years old. The target population will reflect the demographics of the community at large in the area surrounding the study site. Healthy adults, both males and non-pregnant females, will be recruited from the Baltimore/Washington, DC, area through the WRAIR CTC by the use of advertisement in multiple media formats, to include but not limited to: newspapers, fliers, e-mails, the WRAIR CTC web site, public listservs, social media (such as Facebook), posters, bus ads, and generic radio advertisements. E-mail announcements and web site postings (e.g., WRAIR CTC) will include information found on recruitment scripts (excluding any compensation information) or posters excluding any photos unless attached as a complete flyer. Recruitment may also include oral presentations at events, meetings, and briefings wherein the desired recruit population might reasonably be expected to attend. All forms and mechanisms of recruitment as well as the recruitment materials will be approved by the local Institutional Review Board (IRB) prior to use. Retention of participants will be accomplished through study reminder telephone calls, texts, emails and mailings as per the preferences of each individual volunteer.

4.3.2. Inclusion Criteria for Enrollment

The PI or designee will make the final decision of eligibility. Only eligible subjects will be given the investigational product.

Subjects must meet all of the following criteria to be included in the study:

1. Healthy, adult, male or female, age 18 to 50 years (inclusive) at the time of enrollment.
2. Completion and review of comprehension test (achieved $\geq$ 70% accuracy, two attempts allowed).
3. Provide written informed consent before initiation of any study procedures.
4. Agrees to complete all study visits and procedures and provide a screening stool sample.
5. Women of childbearing capacity: Negative pregnancy test with understanding (through informed consent process) to not become pregnant during the study or within three (3) months following the last vaccine dose.

4.3.3. Exclusion Criteria for Enrollment

Subjects meeting any of the following criteria will be excluded from the study.

General Health

1. Health problems (for example, chronic medical conditions such as psychiatric conditions, diabetes mellitus, hypertension or any other conditions that might place the subject at increased risk of adverse events) – study clinicians, in consultation with the PI, will use clinical judgment on a case-by-case basis to assess safety risks under this criterion. The PI will consult with the Research Monitor as appropriate.
2. History of autoimmune disorders, cardiovascular and renal diseases.
3. Use of immunosuppressive medications (systemic corticosteroids or chemo-therapeutics that may influence antibody development), or immunosuppressive illness, including IgA deficiency (defined by serum IgA <7mg/dL).
4. Women who are pregnant or planning to become pregnant during the study period plus 3 months beyond the last vaccine dose and currently nursing women.
5. Participation in research involving another investigational product (defined as receipt of investigational product or exposure to invasive investigational device) 30 days before planned date of first vaccination or anytime through the last in-clinic study safety visit.
6. Positive blood test for HBsAG, HCV, HIV-1/2.
7. Clinically significant abnormalities on basic laboratory screening (see protocol Section 8.1).
8. Systemic antimicrobial treatment (i.e., topical treatments are not an exclusion) within 1 week before administration of the first vaccine.

Research Specific

9. Allergies that may increase the risk of AEs.
10. Regular use (weekly or more often) of antidiarrheal, anti-constipation, or antacid therapy.
11. Abnormal stool pattern (fewer than 3 stools per week or more than 3 stools per day) on a regular basis; loose or liquid stools on other than an occasional basis.
12. Personal or family history of inflammatory arthritis.
13. Positive blood test for HLA-B27 (associated with increased risk of reactive arthritis secondary to *Shigella* infection).
14. History of allergy to any vaccine.
15. Exclusionary skin disease history/finding that would confound assessment or prevent appropriate local monitoring of AEs, or possibly increase the risk of a local AE.

Prior Exposure to *Shigella*

16. Serum IgG titer > 2500 to *Shigella flexneri* 2a LPS.
17. History of microbiologically confirmed *Shigella* infection.
18. Received previous licensed or experimental *Shigella* vaccine or live *Shigella* challenge.
19. Travel to countries where *Shigella* or other enteric infections are endemic (most of the developing world) within two years prior to dosing (clinician judgment).
20. Occupation involving handling of *Shigella* bacteria currently, or in the past 3 years.

4.3.4. Criteria for Removal of a Subject from Therapy or Study Assessments

The PI may discontinue a subject's activity without the subject's consent if any of these criteria is met:

- A subject fails to comply with study procedures
- A subject's safety or health may be compromised by further participation
- It is determined to be in the subject's best interest

If a subject is acutely ill on the day of first vaccination, the subject will not be vaccinated and may be asked to return to be enrolled in a subsequent dose group. If a subject is acutely ill on the day of vaccine Dose 2, the subject may return for vaccination after recovery from the acute illness if the return visit is within the compliance range for receiving that dose.

4.4. Treatments

4.4.1. Treatments Administered

Vaccine

Invaplex$_{AR\text{-}DETOX}$, administered by intramuscular immunization at doses of 2.5, 10 or 25 µg.

Placebo

Placebo (saline) administered by intramuscular immunization.

4.4.2. Identity of Investigational Product

S. flexneri 2a Invaplex$_{AR\text{-}DETOX}$ Lot 1972 was produced under cGMP at the WRAIR Pilot Bioproduction Facility, Silver Spring, MD. The individual components IpaB (Lot 1757), IpaC (Lot 1771) and detoxified LPS (Lot 1902) were assembled into the Invaplex$_{AR\text{-}DETOX}$ complex and subsequently purified by ion-exchange chromatography yielding the bulk drug product (Lot 1954). The drug product, designated as Lot 1972, was filled in 2 mL glass vials, stoppered and sealed, and stored at -80 ± 10°C.

4.4.3. Method of Assigning Subjects to Treatment Groups (Randomization)

Enrollment/randomization is performed through the enrollment module in the electronic data capture system, maintained by the Statistical Data Coordinating Center (SDCC): Emmes. Randomization will be stratified by cohort using a blocking factor of size 5. The allocation ratio for each cohort is 4:1, so each cohort will consist of 4 blocks of 5 subjects, 4 who will receive Invaplex$_{AR\text{-}DETOX}$ and one who will receive placebo, in random order.

The first cohort will be enrolled in a staggered manner in order to evaluate the safety and tolerability of the vaccine in a limited number of subjects before exposing a larger number of subjects to the study product.

The pilot group will assign subjects to each treatment group with a ratio of 4:1, so that 4 subjects will receive Invaplex$_{AR\text{-}DETOX}$ and 1 subject will receive placebo.

After review of the safety data through day 7 of this pilot group, if no safety concerns are identified, the remaining 15 subjects will be randomly assigned to one of the two treatment groups in the same ratio (4:1) with 12 subjects receiving Invaplex$_{AR\text{-}DETOX}$ and 3 subjects receiving placebo.

To guard against loss of statistical power due to subjects who may drop-out between randomization and vaccine administration, provision will be made for replacement subjects to be enrolled. Replacement subjects will receive the same product (in a blinded manner) as the drop-out was scheduled to receive. Subjects who receive at least one vaccination will not be replaced.

The randomization scheme was generated and maintained by the Statistical Data Coordinating Center (SDCC): Emmes, Rockville, MD.

4.4.4. Selection of Doses in the Study

Subjects will receive three doses of either Invaplex$_{AR\text{-}DETOX}$ (2.5, 10, or 25 μg) or placebo.

4.4.5. Selection and Timing of Dose for Each Subject

This is a double-blind, placebo-controlled, dose-escalation study, with subjects randomized in a sequential manner to Cohort A (2.5 μg), B (10 μg), or C (25 μg). Within each cohort a permuted block randomization will be used to assign subjects to vaccine or placebo in a 4:1 ratio, respectively. Each subject will receive three doses at 3-week intervals (days 1, 22 and 43). Initiation of a higher dose cohort will begin only after the previous cohort has completed all 3 doses and all safety data through 7 days post-third dose have been reviewed by the PSRT.

4.4.6. Blinding

This is a double-blind study; study subjects, study personnel who perform study assessments after vaccine administration, data entry personnel at the site, and laboratory personnel (including those performing immunology assays) will be masked to treatment assignment. The Emmes statistician and other designated staff will have access to the unblinded treatment assignments. The study is designed to keep subjects and investigators blinded until completion of the clinical phase of the trial and monitoring of the clinical data. Members of the study staff not involved in clinical outcome assessment will perform formulation of the test articles. During the course of the study, all efforts will be made to keep subjects and investigators unaware of subject assignment.

The vaccine/control will be prepared by the unblinded vaccine formulator who will refer to a Treatment Key Listing, provided for the trial by Emmes, to determine the treatment for the subject. The vaccine formulator will maintain the Treatment Key Listing under locked/secured conditions and will not reveal the randomization code to any other study staff member or subject. The investigational study product prepared by the qualified unblinded research vaccine formulator will be witnessed by another unblinded study staff member then dispensed in a syringe, labeled with subject number, and administered by a blinded clinician. All follow-up safety and efficacy evaluations will be performed by blinded clinic staff.

Randomization data are kept strictly confidential, and should be accessible only to authorized persons, until the time of unblinding.

4.4.6.1. Unblinding Procedure

The site investigator may require that the blind be broken for any subject experiencing an emergency when knowledge of the subject's treatment assignment may be necessary for subsequent clinical care.

Every effort should be made not to unblind the subject unless it is considered necessary for the welfare of the subject. Prior to unblinding, the site Investigator is encouraged (to the extent possible, without jeopardizing the subject's health) to contact the Sponsor (or designee) to discuss the decision to break the blind. The site PI will be expected to provide a rationale for the necessity of unblinding based on the expectation that knowledge of the subject's treatment assignment will have a meaningful impact on the subject's medical care in the short term.

If a subject's treatment assignment is unblinded, the subject will remain in the study and continue with protocol-defined study visits, but not receive further study vaccines. The decision to unblind will be communicated to the regulatory bodies (e.g., institutional review boards [IRBs]) as required. At the end of the study, documentation of all unblinded subjects (and the rationale for unblinding) will be incorporated into the Trial Master File.

Study unblinding will occur after study completion, when all data have been entered, all data queries have been resolved, and the database has been locked.

4.4.7. Prior and Concomitant Therapy

Subjects taking regular medication (i.e., birth control pills) prior to enrollment in the trial will be allowed to continue to take this medication unless it is specifically excluded as part of the inclusion/exclusion criteria for the trial. Subjects needing to take non-approved or excluded medication will not be eligible for enrollment in this study. Investigators will make determinations of continued eligibility throughout the trial. Any medication ordered by the study physician during the course of the trial will be documented on appropriate source documents. Medications being taken prior to and during the course of the trial will also be documented in this manner.

4.4.8. Treatment Compliance

All subjects should receive 3 study vaccinations and the second and third should be within ±2 days of the scheduled visit time. All vaccines will be administered or witnessed by a study investigator, clinical research coordinator, or designee. Each subject will be observed for at least 30 minutes after administration in case of any immediate adverse reactions. If a subject experiences an immediate adverse reaction, he/she will be treated and the event will be recorded in the eCRF.

4.4.9. Protocol Deviations

All subject-specific deviations from the protocol (e.g., failure to return for follow-up visits or blood collection within the time indicated in the protocol) are to be documented. The PI or designee will be responsible for identifying and reporting all deviations, which are defined as isolated occurrences involving a procedure that did not follow the study protocol or Manual of Procedures (MOP). Deviations will be reported annually in the continuing review report to the IRB and HRPO, if appropriate. The PI or sub-investigator will assess action taken in response to the deviation and the impact of the deviation.

Any protocol deviation that adversely affects the safety or rights of a subject or the scientific integrity of the study will be reported immediately to the sponsor and the NMRC IRB.

4.5. Immunogenicity and Safety Variables

The following section describes the collection of immunogenicity and safety variables. For a detailed schedule of activities, refer to **Table 2**. For a list of the primary and secondary immunogenicity and safety variables, refer to Section **3.2** and Section **8** .

4.5.1. Adverse Events

4.5.1.1. Reactogenicity Events

Solicited AEs are pre-specified local and general (systemic) adverse events that are common and known to occur or are of particular interest following administration of the study vaccine. For this trial, solicited AEs will be assessed by study staff 30 minutes after each vaccination and then by study subjects daily for 7 days (day of vaccination and subsequent 7 days). Subjects are provided a diary card for recording the presence or absence and severity of solicited AEs.

Investigators will review diary cards with the subject and document positive findings on source documents or progress notes.

Solicited reactogenicity events include the following:

Local Reactions:

- Site pain, site tenderness, swelling, induration, site redness and pruritus.

Systemic Reactions:

- Fever, nausea, vomiting, abdominal pain, diarrhea (loose stools), appetite change, fatigue, headache, myalgias, arthralgias and malaise.

4.5.1.2. Unsolicited Adverse Event

Refer to Section 3.3.1 in this report or Section 11.5 in the protocol for a more detailed definition of AE. The occurrence of an AE might come to the attention of study personnel during study visits or during interviews of a study subject who presents separately for medical care. Information to be collected on AEs includes event description, time of onset, assessment of severity, relationship to study product (assessed only by the PI), and time of resolution/ stabilization of the event. Unsolicited Adverse Events are non-serious adverse events occurring from the time of each study injection through approximately 28 days after each injection.

4.5.1.3. Serious Adverse Event (SAE)

Refer to Section 3.3.2 in this report for the definition of SAE. SAEs are collected from the time of first study injection through last study visit or last contact.

4.5.2. Severity of Adverse Events

All AEs will be assessed for severity by the investigator. The investigating team will execute the investigator severity scale below. Inherent in this assessment is the medical and clinical consideration of all information surrounding the event including any medical intervention required. Each event will be assigned one of the following categories: mild, moderate, severe, or potentially life-threatening. See Table 4 for vital signs and Table 5 and Table 6 for laboratory values for further guidance in the assignment of severity. The following criteria may be used for any symptom not included in the grading scale. The eCRF for AEs will reflect only the highest severity for continuous days an event occurred.

• **Mild** (Grade 1)	Does not interfere with routine activities Minimal level of discomfort
• **Moderate** (Grade 2)	Interferes with routine activities Moderate level of discomfort
• **Severe** (Grade 3)	Unable to perform routine activities Significant level of discomfort
• **Potentially life-threatening** (Grade 4)	Hospitalization or ER visit for potentially life-threatening event

FDA guidelines for toxicity will be followed; however, if a subject is evaluated in an emergency room for a non-life-threatening illness or symptoms (i.e., visits emergency department on weekend for mild problems because the physician's office is closed), the information from that visit will be reviewed, and severity of the AE will be assessed according to the subject's clinical signs and symptoms.

4.5.3. Immunogenicity Variables

Immunological analyses will be performed from blood samples drawn at follow up clinic appointments per the schedule in Table 2 to assess the immunologic responses to IM administration of $Invaplex_{AR\text{-}DETOX}$: 1) serum samples for antibody (IgG and IgA) titers against Invaplex; and 2) (IgG and IgA) titers against Invaplex from ALS. Reciprocal endpoint titers less than the starting dilution (LLOQ) of the assay will be assigned a value of half the starting dilution for computational purposes. In all statistics and listings, values <LLOQ will be presented as "<LLOQ", with LLOQ replaced with the actual starting dilution.

5. SAMPLE SIZE CONSIDERATIONS

A total of 60 subjects are planned for this study. The sample size for this study was limited by the early stage (Phase 1) of the product concept/testing and was designed to evaluate preliminary safety data but not designed to show statistically significant differences between groups. Given the small number of subjects per group, the precision of the estimate for AEs is limited. For example, using binomial probability formulae for no observed adverse events within the 16 subjects receiving a particular dose of the active vaccine yields a 95% CI of 0-21%.

6. GENERAL STATISTICAL CONSIDERATIONS

6.1. General Principles

All analyses will be grouped by dose (2.5, 10, 25 µg, placebo). In general, all data will be listed, sorted by dose and subject, and when appropriate by visit number within subject. All summary tables will be structured with a column for each treatment group in order of increasing dose, with placebos pooled at the end, and will be annotated with the total population size relevant to that table, including any missing observations.

6.2. Timing of Analyses

Blinded safety data through seven days after the third dose in each cohort will be prepared and reviewed by the Protocol Safety Review Team (PSRT) prior to enrollment into the next cohort. For details, refer to Section 6.6.

Once the full set of samples for key immunogenicity variables have been collected at the day 71 visit (28 days after the third vaccination), the database will be frozen for an unblinded (at the group level) topline results analysis.

A final analysis of all data collected through day 223 (by telephone) will be performed after all data queries have been resolved and the data base locked.

6.3. Analysis Populations

A summary of the analysis populations by study group will be prepared (**Table 8**, **Appendix A**).

6.3.1. Safety Population

The safety population will consist of all subjects who received at least one immunization. Subjects will be grouped according to the actual product received. This population will be used for all safety analyses. Denominators for different safety endpoints may vary according to the number of subjects with available data for the specific endpoint.

6.3.2. Immunogenicity (IMM) Population

The immunogenicity population will be adapted to each analysis time point and parameter, and will include all subjects who received at least two vaccinations, who have baseline and post-vaccination data for the immunogenicity variable of interest. Subjects with missing baseline data, major protocol deviations prior to the analysis time point, that are likely to affect immunology results, or who only received a single vaccine dose, will be excluded.

6.4. Covariates and Subgroups

The protocol does not define any formal subgroup analyses, and the study is not adequately powered to perform subgroup analyses.

6.5. Missing Data and Outliers

All attempts will be made to collect all data per protocol. As missing data are expected to be minimal, no imputation will be performed for missing values. Any data point that appears to be erroneous or inexplicable based on clinical judgment will be investigated as a possible outlier. If data points are identified as outliers, sensitivity analyses will be performed to examine the impact of including or excluding the outliers. Any substantive differences in these analyses will be reported. Non-analyzable data will be documented in the deviations.

6.6. Protocol Safety Review Team (PSRT)

No formal interim analyses involving hypothesis testing were planned. The analyses described here are for safety review only, by the PSRT.

A PSRT will be established by PATH to monitor the study and to provide independent, non-binding advice on safety and ethics. The PSRT will be composed of the Principal Investigator, the PATH Medical Officer and the Independent Research Monitor who will periodically review the conduct and safety of the study. The PSRT will be supported by an unblinded secretary and an unblinded biostatistician, both from Emmes. The responsibilities and procedures of the PSRT are defined in the PSRT Charter.

Three blinded reviews of data will be conducted by the PSRT when safety, reactogenicity and clinical lab data from all subjects in a cohort are available up to 7 days post-dose 3 of the study vaccine. Each report will be provided to the PSRT for their review to determine whether the study can continue to enroll for the next, dose-escalating cohort or whether the study should be stopped. At the time of each interim analysis, PATH will neither have access to the individual treatment assignments, nor to any unblinded safety reports (if applicable), but will be provided with aggregated safety results per cohort to allow strategic decisions for the future of the study.

The following summaries will be generated by group and all analyses will be performed on the safety population. Each PSRT report will be based on a unique frozen data base.

1. Occurrence of solicited local AEs during a 7-day follow-up period (i.e., on the day of vaccination and 7 subsequent days) after the first, second and third dose.
2. Occurrence of solicited systemic AEs during a 7-day follow-up period (i.e., on the day of vaccination and 7 subsequent days) after the first, second and third dose.
3. Occurrence of unsolicited AEs after each dose and up to 28 days after the third dose (dose intervals are 21 days).
4. Occurrence of hematological (red blood cell, white blood cell, differentials, hematocrit, platelet count and hemoglobin) and biochemical (Sodium, Potassium, Glucose, AST, ALT, BUN and Creatinine) laboratory abnormalities within 7 days of each dose.
5. Occurrence of SAEs at any time during the study at the time of data freeze.

6.7. Multiple Comparisons/Multiplicity

Due to the early clinical trial phase and the exploratory nature of this study, no adjustment for multiplicity will be performed.

7. STUDY SUBJECTS

7.1. Disposition of Subjects

Table 7 will present a summary of the reasons that subjects were screened but not enrolled. The composition of analysis populations, including reasons for subject exclusion, by study group, will be presented in Table 8. The disposition of subjects and receipt of study vaccinations will be tabulated by study group for all subjects. Summary of subject disposition will include number of subjects screened, enrolled, receiving study product, completing the study, and with immunogenicity results available (Table 9). A CONSORT diagram of the study will also be prepared (Figure 1).

A listing of subjects who discontinued vaccinations or terminated from study follow-up and the reason will be included in Listing 1.

7.2. Protocol Deviations

A summary of subject-specific protocol deviations will be presented by the deviation category, deviation type, and study group for all subjects (Table 3). All subject-specific protocol deviations and non-subject-specific protocol deviations will be included as data listings (Listing 2 and Listing 3). Protocol deviations will not necessarily always lead to exclusion from the Immunology analysis population.

8. SAFETY EVALUATION

Safety is the primary objective of this study. Continuous measures will be assessed using mean, standard deviation, median, and range; categorical measures will be assessed using frequencies and proportions with exact 95% CIs. For safety assessments presented by time point, unscheduled assessments will be summarized as separate time points in chronological order with scheduled study visits.

All safety analyses will be based on the safety population and presented by treatment group.

8.1. Demographic and Other Baseline Characteristics

A summary table of continuous measures (age, height, weight) and categorical measures (gender, race, ethnicity) will be presented by treatment group and overall (Table 10). Demographic listing will also be prepared (Listing 4).

8.1.1. Prior and Concurrent Medical Conditions

All current illnesses and past pre-existing medical conditions will be MedDRA® coded using MedDRA dictionary version 20.1 or higher. Summaries of subjects' pre-existing medical conditions will be prepared (Table 11), and individual subject listings will be prepared for all pre-existing medical conditions (Listing 5).

8.1.2. Prior Medications

A summary of medications that were taken strictly prior to dosing will be presented by WHO Drug Classification (**Table 12**). Individual subject data will be included in the listing prepared for all concomitant medications (**Listing 14**).

8.2. Measurements of Treatment Compliance

A summary of the number of doses of study product administered to subjects will be prepared as part of the subject disposition table (**Table 9**, **Appendix A**).

8.3. Adverse Events

The primary objective of this study is safety of the $Invaplex_{AR\text{-}DETOX}$ vaccination. All summaries of adverse events will be presented by vaccine dose (1 to 3) and overall, and by treatment group. Safety summaries and analyses will be presented for the Safety Population.

Local and general (systemic) solicited AEs, as well as lymph node assessments, are collected within 30 minutes of each vaccine dose and through 7 days following each dose (day of vaccination and subsequent 7 days). Lymph nodes are assessed as palpable or not palpable and for tenderness (yes/no). All other solicited events and symptoms are graded as 0 (normal), 1 (mild), 2 (moderate), 3 (severe), and 4 (potentially life-threatening). All general and local reactions are listed below.

Local Reactions:

- Site pain, site tenderness, swelling, induration, site redness and pruritus.

General Reactions:

- Fever, nausea, vomiting, abdominal pain, diarrhea (loose stools), appetite change, fatigue, headache, myalgias, arthralgias and malaise.

Adverse events will be summarized by MedDRA® system organ class (SOC), preferred term (PT), severity (mild, moderate, severe, potentially life-threatening) and whether related (definitely, probably or possibly) or not related (unrelated, unlikely) to study product. When calculating the incidence of adverse events (i.e., on a per subject basis), each subject will only be counted once per category. Per protocol, all adverse events occurring within 28 days of the third vaccination (Day 71) will be presented (**Table 18**, **Appendix A** and **Listing 10**, **Appendix C**). All events reported on the adverse event CRF will be included in the listing.

To assess safety, the number and percentage of subjects experiencing at least one AE, and the number and percentage of subjects experiencing each specific AE, categorized by body system, preferred term and product administered (for individual post-dose periods and for the entire study period) along with exact 95% CIs, will be tabulated. Overall summaries by study group include the number and percentage of subjects experiencing: (1) any adverse experience; (2) any Grade 2 or greater AE; (3) any AE judged related to study product; (4) any Grade 2 or greater AE judged related to study product; (5) Any SAE; (6) Any SAE related to IP.

Rates of all adverse events will be analyzed by Pearson's Chi-square test (or Fisher's exact test if assumptions are not met for Pearson's Chi-square), to compare groups, if applicable.

A listing of all AEs by subject will be presented (**Listing 10**, **Appendix C**).

The following summaries of adverse events will be presented by SOC, PT, study product and vaccination number:

- Total frequency of AEs (**Table 19** and **Table 20**).
- Subject level summaries of severity and relationship to study product (**Table 21** through **Table 28**).
- Listing of Grade 2 or greater non-serious AEs (**Table 29**).

8.4. Deaths, Serious Adverse Events and Other Significant Adverse Events

Deaths, SAEs and other significant AEs will be presented (**Table 31**, **Appendix A**), including Subject ID, Age (years), Event Description, Onset Date/End Date, Last Dose Received/Days Post Dose, Reason Reported as an SAE, Relationship to Treatment, Alternate Etiology if Not Related, Action Taken, Outcome, and Duration of Event (days). If a reasonable number of events is reported then a subject level summary will be presented.

8.5. Pregnancies

For any subjects in the Safety population who become pregnant during the study, every attempt will be made to follow them through completion of pregnancy to document the outcome, including information regarding any complications with pregnancy and/or delivery. Listings of pregnancies and outcomes will be presented (**Listing 15**, **Appendix C**).

8.6. Clinical Laboratory Evaluations

The distribution of each laboratory value will be presented by time point and treatment group (**Table 31**, **Appendix A**), including mean, standard deviation, mean change, median, median change, and range. The severity and relationship to study product of each laboratory value will be summarized separately (**Table 32**, **Appendix A**). A listing of all values in subjects will be prepared (**Listing 11** and **Listing 12**, **Appendix C**).

8.7. Vital Signs and Physical Evaluations

Vital sign measurements including systolic blood pressure (mmHg), diastolic blood pressure (mmHg), oral temperature (°C or °F) and heart rate (beats/minute) will be assessed at each clinic visit. Between group differences in heart rate, systolic and diastolic blood pressure will be compared using the nonparametric Kruskal-Wallis test, unless normality assumptions are fulfilled for ANOVA. Within-group changes from baseline will be assessed using the signed-rank test, unless normality assumptions are fulfilled for the paired t-test. Normality assumptions will be assessed using goodness-of-fit tests based on the empirical distribution function and by inspection of normal probability plots. Vital signs will be tabulated by visit and treatment group (**Table 33**, **Appendix A**), including mean, standard deviation, median and range and a full listing will be prepared (**Listing 13**, **Appendix C**).

A complete physical examination will occur at screening, followed by a brief physical exam about 7 days prior to the first vaccination. If the initial screening is within the day -7 window (-14 to -2), it can count as both the initial screening and pre-vaccination visit. A table summarizing the occurrence of abnormal physical exam findings will be presented by body system and treatment group (**Table 34**, **Appendix A**). A listing of physical exam values, indicating any abnormalities, will be presented (**Listing 13**, **Appendix C**).

8.8. Concomitant Medications

Concomitant medications will be coded to the Anatomical Therapeutic Classification using the WHO Drug Dictionary. The use of prior and concomitant medications taken during the study will be recorded on the CRFs. The use of concomitant medications during the study will be summarized by ATC1, ATC2 code and study group for the Safety population (**Table 12**, **Appendix A**) and a by-subject listing of prior and concomitant medication use will be prepared (**Listing 14**, **Appendix C**).

9. IMMUNOGENICITY

The analysis of immunogenicity will be performed on the Immunogenicity Population.

In general, descriptive statistics (mean and SD of $\log_{10}$ titers, GMT and 95% CI, median, range) will be tabulated by treatment group and time-point. The two-sided 95% CI will be obtained using a *t*-distribution. Additionally, the geometric mean fold-rise from baseline (GMFR) will be computed (based on the difference in log titer of post-baseline measurement minus baseline) and summarized in the same manner. For GMTs and GMFRs, between-group comparisons will be examined with ANOVA. The normality of the log-transformed continuous outcomes will be assessed using goodness-of-fit tests based on the empirical distribution function and by inspection of the normal probability plot. If normality assumptions are not satisfied, then the Kruskal-Wallis test will be used.

The number and proportion of responders (subjects who seroconvert, i.e., $\geq$ 4-fold increase in endpoint titer between baseline and post-vaccination samples), together with exact Clopper-Pearson 95% CIs will be tabulated by treatment group and time-point. If appropriate, between groups comparisons will be examined with Fisher's exact test unless assumptions are fulfilled for the χ^2 test.

In addition to the tables, reverse cumulative distribution (RCD) curves will be presented by parameter, visit and treatment group, for both GMTs and GMIs. For each parameter at each visit, the treatment groups will be presented by 4 RCD curves.

All statistical tests will be interpreted in a two-tailed fashion using $p<0.05$ to represent statistical significance.

A listing of all immunogenicity results is provided in **Appendix C**, **Listing 6** through **Listing 12**.

9.1. Primary Immunogenicity Analysis

The immunogenicity endpoints listed in Section 3.2.2 will be summarized as above in Table 14 through Table 17, and in Figure 2 through Figure 9 to meet the secondary objective of assessing IgG and IgA serologic and ALS responses to *S. flexneri* 2a Invaplex. Complete listings will be presented in Listing 6 (serum IgA), Listing 7 (serum IgG), Listing 8 (ALS IgA) and Listing 9 (ALS IgG) in Appendix C.

9.2. Exploratory Immunogenicity Analyses

Analysis of exploratory endpoints is beyond the scope of this SAP.

10. REPORTING CONVENTIONS

P-values will be reported to 3 decimal places; p-values less than 0.001 will be reported as "<0.001" and p-values greater than 0.999 will be reported as ">0.999". The median (except for ties), minimum and maximum will be reported on the same scale as the original data. The mean, standard deviation and CIs will be reported to one additional decimal place. Percentages will be reported to one decimal and corresponding 95% CIs will be to two decimals.

11. TECHNICAL DETAILS

SAS version 9.3 or above will be used to generate all tables, figures and listings.

12. SUMMARY OF CHANGES IN THE CONDUCT OF THE STUDY OR PLANNED ANALYSES

No changes have been made at this time.

13. REFERENCES

Not applicable.

APPENDICES

APPENDIX A. TABLES

LIST OF TABLES

A.1 Demographics Tables

9.1 Overall Study Design and Plan Description

Table 1: Study Design [In-Text]

Cohort	Group	N	Route	Study Dose	Timing of Injections
A	A-1	16	IM	$Invaplex_{AR\text{-}DETOX}$ 2.5 µg	Day 1, 22, 43
	A-2	4	IM	Placebo	Day 1, 22, 43
B	B-1	16	IM	$Invaplex_{AR\text{-}DETOX}$ 10 µg	Day 1, 22, 43
	B-2	4	IM	Placebo	Day 1, 22, 43
C	C-1	16	IM	$Invaplex_{AR\text{-}DETOX}$ 25 µg	Day 1, 22, 43
	C-2	4	IM	Placebo	Day 1, 22, 43

IM = intramuscular.

Immunogenicity and Safety Measurements Assessed

Table 2: Clinical Protocol Time and Events Schedule [In-Text]

Visit Number	00A	00B	01	02	03	04	05	06	07	08	09	10	11	12
Study Event	**Screening**	**-7[1]**	**1**	**2**	**8**	**22**	**23**	**29**	**43**	**44**	**50**	**57**	**71[2]**	**223**
Post-Dose Day			**0**	**1**	**7**	**21/0**	**1**	**7**	**21/0**	**1**	**7**	**14**	**28**	**180**
Compliance Ranges (days)	-60 to -9	-14 to -2	N/A	N/A	±1	±2	N/A	±2	±2	N/A	±3	±3	±4	± 28
Study Briefing	X	X												
Comprehension Assessment	X													
Informed Consent (Study Participation)	X													
Informed Consent (HIV Testing)	X													
Screening Medical History/ Physical Exam	X	X												
CBC and Serum Chemistry[3]	X	X			X			X			X			
C-Reactive Protein			X	X	X									
Anti-HIV-1/2		X												
HLA-B27	X													
HBs Ag		X												
Anti-HCV		X												
Serum IgA	X													
Anti LPS Antibody Screening	X													
Vital signs (BP, HR, T)	X	X	X	X	X	X	X	X	X	X	X	X	X	
Urine pregnancy test	X		X			X			X				X	
Dose			**X**			**X**			**X**					
Clinical Check[4]	X	X	X	X	X	X	X	X	X	X	X	X	X	
Symptom Diary[5]			X	X	X	X	X	X	X	X	X			
Serology[6]			X			X			X		X	X	X	
Peripheral Blood Mononuclear Cells (PBMCs)[7]		X	X		X			X			X		X	
Fecal IgA		X	X					X			X			
Additional Sample Collections[8]		X	X					X			X			
Study Completion													X	
Post-Study Safety Assessment[9]														X
Blood Volume (mL) by Study Day	25	82	74	4	70	10	0	67	10	0	77	10	70	0

[1] Day -7 stool range is -14 to -2.
[2] The data will be locked following entry of the Day 71 data
[3] Chemistry includes serum electrolytes, glucose, BUN, creatinine, AST, and ALT. CBC and chemistry will not be repeated if the screening visit occurs during the Day -14 to -9 window.
[4] Clinical checks on dose days include pre- and post-dose complete assessments including targeted physical exams (volunteers will be observed for 30 minutes post-dose), baseline exam, and physical assessment.
[5] Volunteer diaries post-dose will begin day of dose through the 7-day post-dose follow-up for each dose.
[6] Samples taken from blood draw for that day, serum samples will be assayed for antibody (IgG and IgA) titers against *S. flexneri* 2a LPS, IpaB, IpaC, and *S. flexneri* 2a Invaplex by previously established methods.
[7] PBMCs include collection for Antibody Secreting Cells (ASC), Antibody Lymphocyte Supernatant (ALS), memory B and T cells and α4β7+ B cells. A missed PBMC collection on Day -7 will not be considered a protocol deviation.
[8] Additional sample collections may include saliva.
[9] Day 223 assessments will be performed via telephone.

10.2 Protocol Deviations

Table 3: Distribution of Protocol Deviations by Category, Type, and Treatment Group[In-Text]

Category	Deviation Type	Invaplex$_{AR}$ 2.5 µg (N=X)		Invaplex$_{AR}$ 10 µg (N=X)		Invaplex$_{AR}$ 25 µg (N=X)		Placebo (N=X)		All Subjects (N=X)	
		No. of Subj.	No. of Dev.	No. of Subj.	No. of Dev.	No. of Subj.	No. of Dev.	No. of Subj.	No. of Dev.	No. of Subj.	No. of Dev.
Eligibility/enrollment	Any type	x	x	x	x	x	x	x	x	x	x
	Did not meet inclusion criterion										
	Met exclusion criterion										
	ICF not signed prior to study procedures										
	Other										
Treatment administration schedule	Any type										
	Out of window visit										
	Missed visit/visit not conducted										
	Missed treatment administration										
	Delayed treatment administration										
	Other										
Follow-up visit schedule	Any type										
	Out of window visit										
	Missed visit/visit not conducted										
	Other										
Protocol procedure/assessment	Any type										
	Incorrect version of ICF signed										
	Blood not collected										
	Urine not collected										
	Other specimen not collected										

Category	Deviation Type	Invaplex$_{AR}$ 2.5 µg (N=X)		Invaplex$_{AR}$ 10 µg (N=X)		Invaplex$_{AR}$ 25 µg (N=X)		Placebo (N=X)		All Subjects (N=X)	
		No. of Subj.	No. of Dev.	No. of Subj.	No. of Dev.	No. of Subj.	No. of Dev.	No. of Subj.	No. of Dev.	No. of Subj.	No. of Dev.
	Too few aliquots obtained										
	Specimen result not obtained										
	Required procedure not conducted										
	Required procedure done incorrectly										
	Study product temperature excursion										
	Specimen temperature excursion										
	Other										
Treatment administration	Any type										
	Required procedure done incorrectly										
	Study product temperature excursion										
	Other										
Blinding policy/procedure	Any type										
	Treatment unblinded										
	Other										

12.2.2 Displays of Adverse Events

Table 4: Reference Ranges and Adverse Event Coding for Vital Sign Parameters

Parameter	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Heart Rate				
Tachycardia	101–115	116–130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia	50–54[a]	45–49	< 45	ER visit or hospitalization for arrhythmia
Fever (°C) (°F)	38.0–38.4 100.4–101.1	38.5–38.9 101.2–102.0	39.0–40 102.1–104	> 40 > 104
Blood Pressure				
Hypertension (systolic, mm Hg)	141–150	151–155	> 155	ER visit/hospitalization for malignant hypertension
Hypertension (diastolic, mm Hg)	91–95	96–100	> 100	ER visit/hospitalization for malignant hypertension
Hypotension (systolic, mm Hg)[b]	85–89	80–84	< 80	ER visit/hospitalization for hypotensive shock

a Grade 1 bradycardia will not be considered an abnormality for this study unless judged to be clinically significant by the PI or the PI in consultation with the research monitor and sponsor.

b If a subject has a baseline systolic blood pressure in the 90s then a decrease in blood pressure < 10 without associated clinical symptoms will not be considered an abnormality for this study unless judged to be clinically significant by the PI.

If a subject visits an emergency room for a non-life-threatening illness or symptoms (i.e., visits emergency room on weekend for mild problems because the physician's office is closed), the severity of the AE will be assessed according to the subject's clinical signs and symptoms.

Table 5: Reference Ranges and Adverse Event Coding for Clinical Hematology Parameters

Parameter	Quest Normal	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (g/dL) (for screening purposes only)	M: LLN = 13.2 F: LLN = 11.7	M: 12.5-13.1 F: 11.0-11.6	M: 10.5-12.4 F: 9.5-10.9	M: 8.5-10.4 F: 8.0-9.4	M: <8.5 F: <8.0
Hemoglobin - decrease from lower limit of normal (used to grade toxicity)[a]		0.5-1.5	1.6-2.0	2.1-5.0	> 5.0
Neutrophils (cells/mm^3)	1,500-7,800	1,225-1,499	1,000-1,224	776-999	< 776
Leukocytes (white blood cells) (cells/mm^3)	3,800-10,800				
Leukopenia		2,500-3,799	1,500-2,499	1,000-1,499	< 1,000
Leukocytosis		10,801-15,000	15,001-20,000	20,001-25,000	> 25,000
Lymphocytes (cells/mm^3)	850-3,900	750-849	500-749	250-499	< 250
Eosinophils (cells/mm^3)	15-500	551-1,500	1,501-5,000	> 5,000	Hypereosinophilic
Platelets decreased ($10^3/mm^3$)	140-400	125-139	100-124	25-99	< 25

[a] In an instance where a post-vaccination hemoglobin result is below the Quest normal range, an AE is recorded when the post-vaccination value represents a decrease of ≥0.5 g/dL from that subject's baseline value. If a subject has a decrease in hemoglobin ≥0.5 g/dL but remains within the Quest normal range, that will not be documented as an AE.

Table 6: Reference Ranges and Adverse Event Coding for Blood Chemistry Parameters

Parameter	Quest Normal	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life-Threatening (Grade 4)
Sodium	135-146 (mmol/L)				
Hyponatremia		132-134	130-131	125-129	< 125
Hypernatremia		147-148	149-150	151-152	> 152
Potassium	3.5-5.5 (mmol/L)				
Hypokalemia		3.3-3.4	3.1-3.2	2.9-3.0	< 2.9
Hyperkalemia		5.6-5.7	5.8-5.9	6.0-6.1	≥ 6.2
Glucose, Random	65-139 (mg/dL)				
Hyperglycemia		140-155	156-200	> 200	Insulin requirements or hyperosmolar coma
Hypoglycemia		60-64	55-59	45-54	< 45
SGOT/AST (elevation)	M: 10-40 U/L F: 10-30 U/L	M: 41-100 F: 31-75	M: 101-200 F: 76-150	M: 201-400 F: 151-300	M: > 400 F: > 300
SGPT/ALT (elevation)	M: 9-60 U/L F: 6-40 U/L	M: 61-150 F: 41-100	M: 151-300 F: 101-200	M: 301-600 F: 201-400	M: > 600 F: > 400
BUN (elevation)	7-25	26-28	29-31	> 31	Requires dialysis
Creatinine (elevation)	M: 0.7-1.4 F: 0.5-1.1	M: 1.5-1.7 F: 1.2-1.7	M: 1.8-2.0 F: 1.8-2.0	M: 2.1-2.5 F: 2.1-2.5	M: >2.5 F: >2.5 or requires dialysis

14.1 Description of Study Subjects

Table 7: Ineligibility Summary of Screen Failures [In-Text]

Inclusion/Exclusion Category	Inclusion/Exclusion Criterion	n[a]
Inclusion and Exclusion	Subjects failing any eligibility criterion	x
Inclusion	Any inclusion criterion	x
	[inclusion criterion 1]	x
	[inclusion criterion 2]	x
	[inclusion criterion 3]	x
Exclusion	Any exclusion criterion	x
	[exclusion criterion 1]	x
	[exclusion criterion 2]	x
	[exclusion criterion 3]	x
Eligible but not enrolled		x

[a] More than one criterion may be marked per subject.

[To include all inclusion/exclusion criteria]

Table 8: Analysis Populations by Treatment Group

Reason for Exclusion		Invaplex$_{AR}$ 2.5 µg (N=X)	Invaplex$_{AR}$ 10 µg (N=X)	Invaplex$_{AR}$ 25 µg (N=X)	Placebo (N=X)	All Subjects (N=X)
Safety Population		**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Subjects Included						
Received First Vaccination						
Received Second Vaccination						
Received Third Vaccination						
Immunogenicity Populations*		**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Assay	**Completed Visit and Data Available**					
Serum IgA	Day 22 (3 wks post-vac.1)					
	Day 43 (3 wks post-vac.2)					
	Day 50 (1 wk post-vac.3)					
	Day 57 (2 wks post-vac.3)					
	Day 71 (4 wks post-vac.3)					
Serum IgG	Day 22 (3 wks post-vac.1)					
	Day 43 (3 wks post-vac.2)					
	Day 50 (1 wk post-vac.3)					
	Day 57 (2 wks post-vac.3)					
	Day 71 (4 wks post-vac.3)					
ALS IgA	Day 8 (1 wk post-vac.1)					
	Day 29 (1 wk post-vac.2)					
	Day 50 (1 wk post-vac.3)					
	Day 71 (4 wks post-vac.3)					
ALS IgG	Day 8 (1 wk post-vac.1)					
	Day 29 (1 wk post-vac.2)					
	Day 50 (1 wk post-vac.3)					
	Day 71 (4 wks post-vac.3)					

* Subjects with baseline and post-vaccination data, who received all required vaccinations prior to visit.

Table 9: Subject Disposition by Treatment Group

Subject Disposition	Invaplex$_{AR}$ 2.5 µg	Invaplex$_{AR}$ 10 µg	Invaplex$_{AR}$ 25 µg	Placebo	Total
	n	n	n	n	n
Screened*	na	na	na	na	
Enrolled/Randomized					
Received First Dose					
Received Second Dose					
Received Third Dose					
Received all 3 Doses					
Completed Visit 11 (Day 71)					
Completed Visit 12 (Day 223)					
Serology IgA or IgG Results Available					
Visit 01 (Baseline)					
Visit 04 (Pre-Dose 2)					
Visit 07 (Pre-Dose 3)					
Visit 09 (Day 7 post-vac.3)					
Visit 10 (Day 14 post-vac.3)					
Visit 11 (Day 28 post-vac.3)					
ALS IgA or IgG Results Available					
Screening					
Visit 01 (Baseline)					
Visit 03 (Day 7 post-vac.1)					
Visit 06 (Day 7 post-vac.2)					
Visit 09 (Day 7 post-vac.3)					
Visit 11 (Day 28 post-vac.3)					
Fecal IgA Results Available					
Screening					
Visit 01 (Baseline)					
Visit 06 (Day 7 post-vac.2)					
Visit 09 (Day 7 post-vac.3)					

* Screening occurs prior to group assignment, which occurs at randomization.
na = not applicable.

Table 10: Demographics and Baseline Characteristics by Treatment Group - All Enrolled and Randomized Subjects [In-Text]

	Invaplex$_{AR}$ 2.5 µg (N=X)	Invaplex$_{AR}$ 10 µg (N=X)	Invaplex$_{AR}$ 25 µg (N=X)	Placebo (N=X)	All Subjects (N=X)
Characteristic/ Statistics	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
Sex					
Male					
Female					
Ethnicity					
Not Hispanic or Latino					
Hispanic or Latino					
Not Reported					
Unknown					
Race					
American Indian or Alaska Native					
Asian					
Native Hawaiian or Other Pacific Islander					
Black or African American					
White					
Multi-Racial					
Unknown					
Age (years)					
Mean (SD)					
Median					
Min/Max					
Weight (lbs)					
Mean (SD)					
Median					
Min/Max					
Height (ins)					
Mean (SD)					
Median					
Min/Max					

Table 11: Pre-Existing Medical Conditions by MedDRA® System Organ Class and Treatment Group

	Invaplex$_{AR}$ 2.5 µg (N=X)		Invaplex$_{AR}$ 10 µg (N=X)		Invaplex$_{AR}$ 25 µg (N=X)		Placebo (N=X)		All Subjects (N=X)	
MedDRA System Organ Class	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Any SOC	x	xx	x	xx	x	xx	x	xx	x	xx
[SOC 1]										
[SOC 2]										

N= Number of subjects enrolled.
n = Number of subjects reporting medical history within the specified SOC.
A subject is only counted once per SOC.

Table 12: Prior and Concomitant Medications, by WHO Drug Classification and Treatment Group

	Invaplex$_{AR}$ 2.5 µg (N=X)		Invaplex$_{AR}$ 10 µg (N=X)		Invaplex$_{AR}$ 25 µg (N=X)		Placebo (N=X)	
	Prior	**Con**	**Prior**	**Con**	**Prior**	**Con**	**Prior**	**Con**
	n (%)	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**	**n (%)**
[ATC Level 1]	x (x.x)	x (x.x)	x (x.x)	x (x.x)	x (x.x)	x (x.x)	x (x.x)	x (x.x)
[ATC Level 2 - 1]								
[ATC Level 2 - 2]								
ETC.								

N= Number of subjects enrolled. n = Number of subjects who reported taking medication.
Prior = Medications taken prior to, but not continuing at enrollment.
Con = Medications taken at any time on or after enrollment.
A subject is only counted once per medication.

A.2 Immunogenicity Tables

Table 13: Number of Immunogenicity Samples Collected and Analyzed[1]

Assay	Invaplex$_{AR\text{-}DETOX}$ Dose	Dose 1		Dose 2		Dose 3			
		Baseline[2]	Day 7	Pre-Dose	Day 7	Pre-dose	Day 7	Day 14	Day 28
Serology									
S. flexneri 2a Invaplex IgA	2.5 µg								
	10 µg								
	25 µg								
S. flexneri 2a Invaplex IgG	2.5 µg								
	10 µg								
	25 µg								
PBMCs for ALS									
S. flexneri 2a Invaplex IgA	2.5 µg								
	10 µg								
	25 µg								
S. flexneri 2a Invaplex IgG	2.5 µg								
	10 µg								
	25 µg								

[1] Analyzed by the lab and data available.
[2] Most recent observation prior to dose 1.
ALS: Antibody Lymphocyte Supernatant; IgA: Immunoglobulin A; IgG: Immunoglobulin G.

A.2.1 Primary Immunology Outcomes

Table 14: Serologic IgA Responses to *S. flexneri* 2a Invaplex, Descriptive Statistics by Study Day and Treatment Group - Immunogenicity Population

Time Point	Statistic	$Invaplex_{AR\text{-}DETOX}$ Dose 2.5 µg (N=X)	$Invaplex_{AR\text{-}DETOX}$ Dose 10 µg (N=X)	$Invaplex_{AR\text{-}DETOX}$ Dose 25 µg (N=X)	Placebo (N=X)
Serum IgA					
Baseline	n	xx			
	Mean	xxx.x			
	Median	xxxx			
	SD	xxx.x			
	Range	xxxx, xxxx			
Day 22 Pre-Dose 2	n				
	Mean				
	Median				
	SD				
	Range				
	GMFR				
	GMFR 95% CI				
Repeat for					
Day 43 Pre-Dose 3					
Day 50 Post-Dose Day 7					
Day 57 Post-Dose Day 14					
Day 71 Post-Dose Day 28					

N = Number of subjects in the immunogenicity population. n = Number of subjects with data at the specified time point. GMFR = Geometric Mean Fold-Rise from Baseline. SD =Standard Deviation. CI = Confidence Interval.

Table with similar in format to **Table 14**:

Table 15: Serologic IgG Responses to *S. flexneri* 2a Invaplex, Descriptive Statistics by Study Day and Treatment Group - Immunogenicity Population

Table 16: Antibody in Lymphocyte Supernatant (ALS) IgA Responses to *S. flexneri* 2a Invaplex, Descriptive Statistics by Study Day and Treatment Group - Immunogenicity Population

Time Point	Statistic	$Invaplex_{AR\text{-}DETOX}$ Dose			Placebo (N=X)
		2.5 µg (N=X)	10 µg (N=X)	25 µg (N=X)	
Serum IgA					
Baseline	n	xx			
	Mean	xxx.x			
	Median	xxxx			
	SD	xxx.x			
	Range	xxxx, xxxx			
Day 8 Post-Dose 1, Day 7	n				
	Mean				
	Median				
	SD				
	Range				
	GMFR				
	GMFR 95% CI				
Repeat for					
Day 29 Post-Dose 2, Day 7					
Day 50 Post-Dose 3, Day 7					
Day 71 Post-Dose 3 Day 28					

N = Number of subjects in the immunogenicity population. n = Number of subjects with data at the specified time point. GMFR = Geometric Mean Fold-Rise from Baseline. SD =Standard Deviation. CI = Confidence Interval.

Table with similar in format to **Table 16**:

Table 17: Antibody in Lymphocyte Supernatant (ALS) IgG Responses to S. flexneri 2a Invaplex, Descriptive Statistics by Study Day and Treatment Group - Immunogenicity Population

Table A1: Serologic Responses to *S. flexneri* 2a Invaplex, Geometric Mean Titer by Study Day and Treatment Group - Immunogenicity Population [In-text]

Note: Tables A1–A4 are in-text. Table numbers will be adjusted when compiling the CSR.

Time Point	Statistic	Invaplex$_{AR\text{-}DETOX}$ Dose 2.5 µg (N=X)	Invaplex$_{AR\text{-}DETOX}$ Dose 10 µg (N=X)	Invaplex$_{AR\text{-}DETOX}$ Dose 25 µg (N=X)	Placebo (N=X)	P-Value
Serum IgA						
Baseline	n	xx				x.xxxx
	GMT	xxxx				
	95% CI	xxxx - xxxx				
Day 22 Pre-Dose 2						x.xxxx
Day 43 Pre-Dose 3						x.xxxx
Day 50 Post-Dose Day 7						x.xxxx
Day 57 Post-Dose Day 14						x.xxxx
Day 71 Post-Dose Day 28						x.xxxx
Serum IgG						
Baseline	n	xx				x.xxxx
	GMT	xxxx				
	95% CI	xxxx - xxxx				
Day 22 Pre-Dose 2						x.xxxx
Day 43 Pre-Dose 3						x.xxxx
Day 50 Post-Dose Day 7						x.xxxx
Day 57 Post-Dose Day 14						x.xxxx
Day 71 Post-Dose Day 28						x.xxxx

GMT = Geometric Mean Titer. CI = Confidence Interval. P-values are from ANOVA tests of differences between groups in mean log titers.

Table A2: Serologic Responses to *S. flexneri* 2a Invaplex, Seroconversion Rates by Study Day and Treatment Group - Immunogenicity Population [In-text]

Time Point	Statistic	Invaplex$_{AR\text{-}DETOX}$ Dose			Placebo (N=X)	P-Value
		2.5 µg (N=X)	10 µg (N=X)	25 µg (N=X)		
Serum IgA						
Day 22 Pre-Dose 2	n	xx				x.xxxx
	Seroconversion %	xx.x				
	95% CI	xx.x, xx.x				
Day 43 Pre-Dose 3						x.xxxx
Day 50 Post-Dose Day 7						x.xxxx
Day 57 Post-Dose Day 14						x.xxxx
Day 71 Post-Dose Day 28						x.xxxx
Serum IgG						
Day 22 Pre-Dose 2						x.xxxx
Day 43 Pre-Dose 3						x.xxxx
Day 50 Post-Dose Day 7						x.xxxx
Day 57 Post-Dose Day 14						x.xxxx
Day 71 Post-Dose Day 28						x.xxxx

Seroconversion defined as ≥4-fold increase in titer from baseline. CI = Exact Clopper Pearson Confidence Interval. P-values are from Fisher's exact 2-tail tests of differences between groups in seroconversion rates.

Table A3: Antibody in Lymphocyte Supernatant (ALS) Responses to *S. flexneri* 2a Invaplex, Geometric Mean Titer by Study Day and Treatment Group - Immunogenicity Population [In-text]

Time Point	Statistic	Invaplex$_{AR-DETOX}$ Dose 2.5 µg (N=X)	Invaplex$_{AR-DETOX}$ Dose 10 µg (N=X)	Invaplex$_{AR-DETOX}$ Dose 25 µg (N=X)	Placebo (N=X)	P-Value
ALS IgA						
Baseline	n	xx				x.xxxx
	GMT	xxxx				
	95% CI	xxxx - xxxx				
Day 8 Post-Dose 1, Day 7						x.xxxx
Day 29 Post-Dose 2, Day 7						x.xxxx
Day 50 Post-Dose 3, Day 7						x.xxxx
Day 71 Post-Dose 3, Day 28						x.xxxx
ALS IgG						
Baseline	n	xx				x.xxxx
	GMT	xxxx				
	95% CI	xxxx - xxxx				
Day 8 Post-Dose 1, Day 7						x.xxxx
Day 29 Post-Dose 2, Day 7						x.xxxx
Day 50 Post-Dose 3, Day 7						x.xxxx
Day 71 Post-Dose 3, Day 28						x.xxxx

GMT = Geometric Mean Titer. CI = Confidence Interval. P-values are from ANOVA tests of differences between groups in mean log titers.

Table A4: Antibody in Lymphocyte Supernatant (ALS) Responses to *S. flexneri* 2a Invaplex, Seroconversion Rates by Study Day and Treatment Group - Immunogenicity Population [In-text]

Time Point	Statistic	Invaplex$_{AR\text{-}DETOX}$ Dose 2.5 µg (N=X)	Invaplex$_{AR\text{-}DETOX}$ Dose 10 µg (N=X)	Invaplex$_{AR\text{-}DETOX}$ Dose 25 µg (N=X)	Placebo (N=X)	P-Value
ALS IgA						
Day 8	n	xx				x.xxxx
	Seroconversion %	xx.x				
	95% CI	xx.x, xx.x				
Day 29						x.xxxx
Day 50						x.xxxx
Day 71						x.xxxx
ALS IgG						
Day 8						x.xxxx
Day 29						x.xxxx
Day 50						x.xxxx
Day 71						x.xxxx

Seroconversion defined as ≥4-fold increase in titer from baseline. CI = Exact Clopper Pearson Confidence Interval. P-values are from Fisher's exact 2-tail tests of differences between groups in seroconversion rates.

A.3 Safety Tables

A.3.1 Adverse Events

Table 18: Summary of Adverse Events, by Study Group - Safety Population [In-Text]

	Invaplex$_{AR}$ 2.5 µg (N=X)		Invaplex$_{AR}$ 10 µg (N=X)		Invaplex$_{AR}$ 25 µg (N=X)		Placebo (N=X)		
	n(%)	95%CI	n(%)	95%CI	n(%)	95%CI	n(%)	95%CI	P-Value[a]
Up to and including Visit 11 (28 days post-Dose 3).									
Any AE									
Any Grade 2 or Greater AE									
Any AE related to IP									
Any Grade 2 or greater AE related to IP									
Any SAE									
Any SAE related to IP									
After Visit 11 and up to Visit 12 (Day 223).									
Any AE									
Any Grade 2 or Greater AE									
Any AE related to IP									
Any Grade 2 or greater AE related to IP									
Any SAE									
Any SAE related to IP									

N=Number of subjects in the Safety Population. CI=Exact (Clopper-Pearson) Confidence Interval. IP=Investigational Product.

AEs include SAEs. Related = Possibly, probably or definitely related to study product.

[a] Pearson's Chi-square test or Fisher's exact 2-tailed test of any difference in proportions between groups. If statistically significant, significant pairwise differences will be based on non-overlapping 95% CIs.

Table 19: Total Number of Adverse Events, Up to and Including Visit 11 (Day 28 Post-Dose 3), by MedDRA® System Organ Class and Preferred Term, and Treatment Group - Safety Population

MedDRA®		Invaplex$_{AR}$ 2.5 µg (N=X)	Invaplex$_{AR}$ 10 µg (N=X)	Invaplex$_{AR}$ 25 µg (N=X)	Placebo (N=X)
System Organ Class (SOC)	Preferred Term (PT)	No. of Events	No. of Events	No. of Events	No. of Events
Any SOC	Any PT				
[SOC 1]	Any PT				
	[PT 1]				
	[PT 2]				
[SOC 2]	Any PT				
	[PT 1]				
	[PT 2]				

N=Number of subjects in the safety population.
Each row may include multiple events per subject, including repeats of the same AE within a subject.

The following table will have the same format as **Table 19**. Note that grouping is by PT only.

Table 20: Total Number of Adverse Events Related to IP, Up to and Including Visit 11 (Day 28 Post-Dose 3), by MedDRA® Preferred Term, and Treatment Group - Safety Population [In-Text – by PT only]

Table 21: Subjects Experiencing AEs, Up to and Including Visit 11 (Day 28 Post-Dose 3), by MedDRA® System Organ Class (SOC), Preferred Term (PT), Severity and Treatment Group - Safety Population

		Invaplex$_{AR}$ 2.5 µg (N=X)		Invaplex$_{AR}$ 10 µg (N=X)		Invaplex$_{AR}$ 25 µg (N=X)		Placebo (N=X)		
	Severity[a]	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	n (%)	95% CI	P-Value[b, c]
Any SOC										
Any Preferred Term	Any Severity	x (xx.x)	(xx.x, xx.x)	x (xx.x)	(xx.x, xx.x)	x (xx.x)	(xx.x, xx.x)	x (xx.x)	(xx.x, xx.x)	x.xxxx
	Mild									
	Moderate									
	Severe									
	Life-Threatening									
[SOC 1]										
Any Preferred Term	Any Severity									
	Mild									
	Moderate									
	Severe									
	Life-Threatening									
[PT 1]	Any Severity									
	Mild									
	Moderate									
	Severe									
	Life-Threatening									
[PT 2]	Any Severity									
	Mild									
	Moderate									
	Severe									
	Life-Threatening									

N=Number of subjects in the Safety Population. CI=Exact (Clopper-Pearson) Confidence Interval.

AE = Adverse events (including SAEs and solicited AEs) that were reported at any time up to and including Visit 11 (28 days post-Dose 3).

[a] Subjects are only counted once per SOC/PT category, based on maximum severity over all AEs reported within each category.

[b] Pearson's Chi-square test or Fisher's exact 2-tailed test of any difference between groups in proportion of subjects with any event.

[c] For statistically significant ($p<0.05$) p-values, significant pairwise differences will be based on non-overlapping 95% CIs.

The following tables will have the same format as **Table 21**.

Table 22: **Subjects Experiencing AEs Related to Study Product Up to and Including Visit 11 (Day 28 Post-Dose 3), by MedDRA® Preferred Term (PT), Severity and Treatment Group - Safety Population [In-Text, by PT only]**

Table 23: **Subjects Experiencing AEs Post-Dose 1 and Prior to Dose 2, by MedDRA® System Organ Class (SOC), Preferred Term (PT), Severity and Treatment Group - Safety Population**

Table 24: **Subjects Experiencing AEs Related to Study Product, Post-Dose 1 and Prior to Dose 2, by MedDRA® System Organ Class (SOC), Preferred Term (PT), Severity and Treatment Group - Safety Population**

Table 25: **Subjects Experiencing AEs Post-Dose 2 and Prior to Dose 3, by MedDRA® System Organ Class (SOC), Preferred Term (PT), Severity and Treatment Group - Safety Population**

Table 26: **Subjects Experiencing AEs Related to Study Product, Post-Dose 2 and Prior to Dose 3, by MedDRA® System Organ Class (SOC), Preferred Term (PT), Severity and Treatment Group - Safety Population**

Table 27: **Subjects Experiencing AEs Up to 28 Days Post-Dose 3, by MedDRA® System Organ Class (SOC), Preferred Term (PT), Severity and Treatment Group - Safety Population**

Table 28: **Subjects Experiencing AEs Related to Study Product, Up to 28 Days Post-Dose 3, by MedDRA® System Organ Class (SOC), Preferred Term (PT), Severity and Treatment Group - Safety Population**

Table 29: Grade 2 or Greater Non-Serious Adverse Events [In-Text]

Treatment	Subject ID	Age at Onset (years)	Adverse Event	Previous Dose #	Onset Day (Duration)	Severity	Relationship to Study Treatment	Alternative Etiology	Action Taken with Study Treatment	Subject Discontinued Due to AE	Outcome	Comments
[SOC 1]												
[PT 1]												
[PT 2]												
[SOC 2]												
[PT 1]												

[Rows will be sorted by SOC, PT, treatment group, subject, onset day]

Table 30: Deaths, Serious Adverse Events and Other Significant AEs [In-Text]

Similar format as Table 29. Additional information on each event may be provided in a narrative provided by the medical monitor.

A.3.2 Clinical Labs

Table 31: Hematology and Serum Chemistry Test Results, Descriptive Statistics by Treatment Group - Safety Population

		Invaplex$_{AR\text{-}DETOX}$			
		2.5 µg	10 µg	25 µg	Placebo
WBC Count (10^3/µL)					
Screening	N				
	Mean (SD)				
	Median				
	Range				
Pre-Vaccination 1	N				
	Mean (SD)				
	Median				
	Range				
Day 7 Post-Vaccination 1	N				
	Mean (SD)				
	Median				
	Range				
	Mean change from baseline (SD)				
	Median change from baseline				
Day 7 Post-Vaccination 2	N				
	Mean (SD)				
	Median				
	Range				
	Mean change from baseline (SD)				
	Median change from baseline				
Day 7 Post-Vaccination 3	N				
	Mean (SD)				
	Median				
	Range				
	Mean change from baseline (SD)				
	Median change from baseline				
Final "on therapy" value	N				
	Mean (SD)				
	Median				
	Range				
	Mean change from baseline (SD)				
	Median change from baseline				

Baseline values are from pre-vaccination 1. If missing, then screening values will be used.

Table will also include:

Hematology

WBC Count (10^6/µL)*	RBC Count (10^3/µL)**	Hemoglobin (g/dL)*
Hematocrit (%)*	Platelet count (10 / µL)	Absolute Neutrophils (cells/ µL)
Absolute Lymphocytes (cells/ µL)	Absolute Monocytes (cells/ µL)	Absolute Eosinophils (cells/ µL)
Absolute Basophils (cells/ µL)		

Chemistry

Sodium (mmol/L)	Potassium (mmol/L)*	Chloride (mmol/L)
BUN (mg/dL)	Creatinine (mg/dL)**	Bicarbonate (mmol/L)
Glucose (mg/dL)	AST (U/L)	ALT (U/L)

[Programming note: * reported to one decimal place, ** 2 decimal places. All others are integers].

Table 32: Final On Therapy Hematology and Serum Chemistry Severity Grading, by Treatment Group and Relationship[1] to IP - Safety Population

		Invaplex$_{AR}$ 2.5 µg (N=X)	Invaplex$_{AR}$ 10 µg (N=X)	Invaplex$_{AR}$ 25 µg (N=X)	Placebo (N=X)
		n (%)	n (%)	n (%)	n (%)
WBC					
All values	Normal				
	Abnormal[2]				
	Mild				
	Moderate				
	Severe				
	Potentially Life-Threatening				
Not Related to IP	Mild				
	Moderate				
	Severe				
Related to IP	Mild				
	Moderate				
	Severe				
[other parameters]					

N=Number of subjects in the safety population.

[1] Relationship is assessed only for mild or greater values reported as adverse events.

[2] Outside the normal range but less than mild.

[See above for complete list of parameters]

A.3.3 Vital Signs

Table 33: Vital Signs: Descriptive Statistics by Visit and Treatment Group - Safety Population

	Pulse Rate (beats/min)				Systolic Blood Pressure (mm Hg)				Diastolic Blood Pressure (mm Hg)			
	Invaplex$_{AR\text{-}DETOX}$				Invaplex$_{AR\text{-}DETOX}$				Invaplex$_{AR\text{-}DETOX}$			
	2.5 µg (N=X)	10 µg (N=X)	25 µg (N=X)	Placebo (N=X)	2.5 µg (N=X)	10 µg (N=X)	25 µg (N=X)	Placebo (N=X)	2.5 µg (N=X)	10 µg (N=X)	25 µg (N=X)	Placebo (N=X)
Baseline[1]												
n												
Mean												
SD												
Median												
Range												
Visit 02 (1 Day post-Dose 1)												
n												
Mean												
SD												
Median												
Range												
Change from Baseline												
n												
Mean												
SD												
Median												
Range												
Continue for all other Visits (days 2, 8, 22, 23, 29, 43, 44, 50, 57, 71)												

A.3.4 Physical Exam

Table 34: Summary of Abnormal Physical Exam Findings Prior to Dose 1 – Safety Population

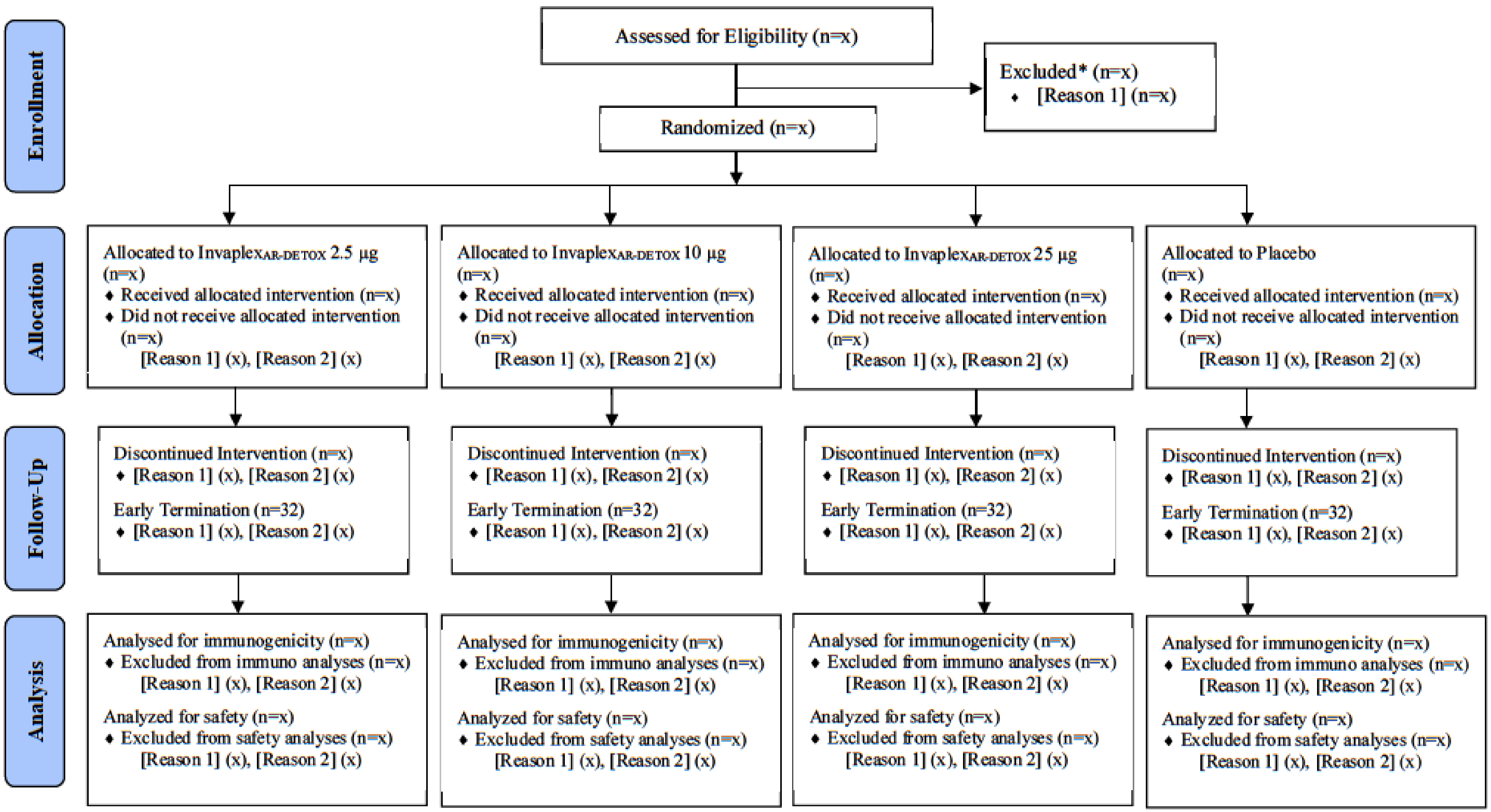
Body System	Visit	Invaplex$_{AR}$ 2.5 µg (N=X)	Invaplex$_{AR}$ 10 µg (N=X)	Invaplex$_{AR}$ 25 µg (N=X)	Placebo (N=X)
Any Abnormality	Screening				
	Day -7				
Skin	Screening				
	Day -7				
HEENT	Screening				
	Day -7				
Lymph Nodes	Screening				
	Day -7				
Respiratory	Screening				
	Day -7				
Cardiovascular	Screening				
	Day -7				
Abdomen	Screening				
	Day -7				
Neurological	Screening				
	Day -7				
Motor	Screening				
	Day -7				
Sensory	Screening				
	Day -7				
Musculoskeletal (Overall)	Screening				
	Day -7				
Musculoskeletal (Joint survey)	Screening				
	Day -7				
Other	Screening				
	Day -7				

APPENDIX B. FIGURES

LIST OF FIGURES

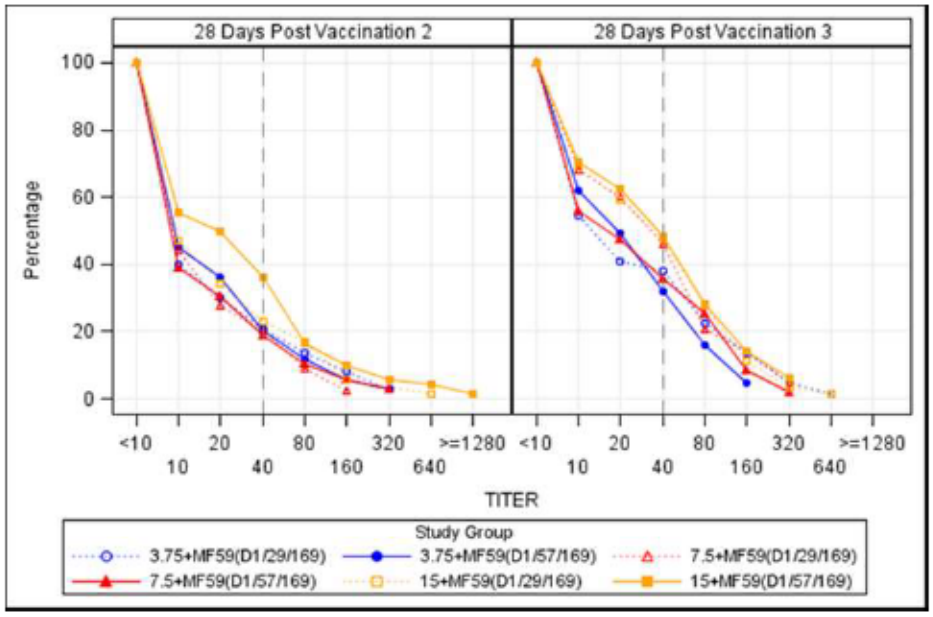
B.1 Demographics Figures

Figure 1: CONSORT Flow Diagram



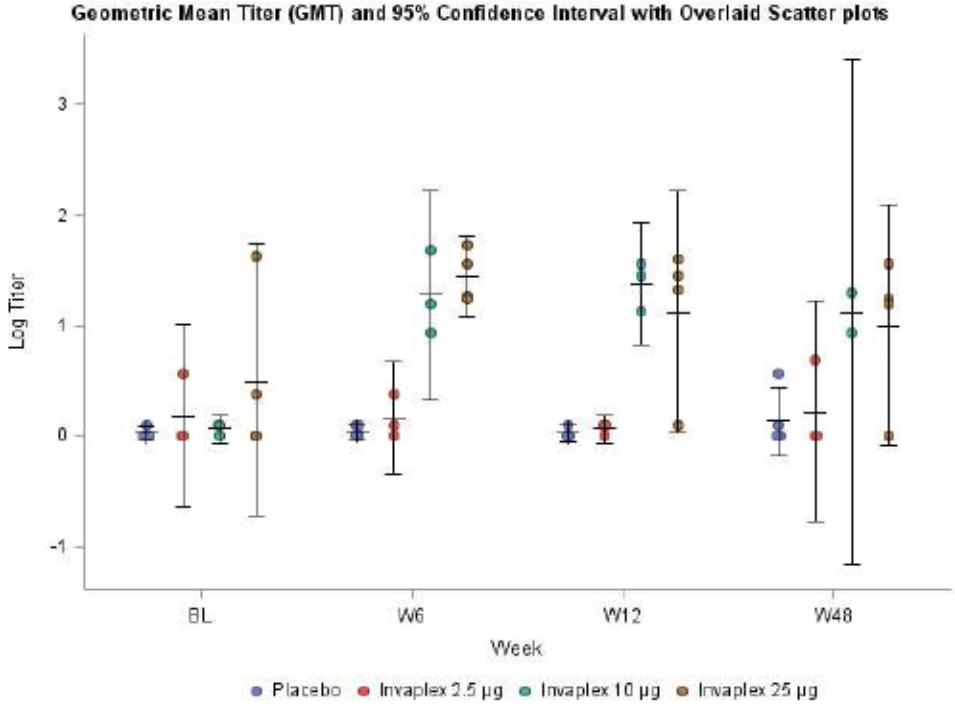
B.2 Immunogenicity Figures

Figure 2: Serologic IgA Responses to *S. flexneri* 2a Invaplex: Distribution of Titers Using Reverse Cumulative Distribution Curves - Immunogenicity Population



Implementation Note: A generic sample figure is shown above. The RCD curves will be presented in a single figure with separate panels for each time point (Visit 01, 04, 07, 09, 10, 11). Visit labels will be included in the panel headers. Within each panel, 4 individual curves will be presented, one for each Study Group. Each curve will be represented by a step function. The legend will indicate study group only.

Figure 3: Serologic IgA Responses to *S. flexneri* 2a Invaplex: Geometric Mean Titer and 95% CI - Immunogenicity Population



Implementation Note: A generic sample figure is shown above. The x-axis will show relevant study visits and be scaled accordingly. All data are plotted on the log-10 scale. For each study group, the GMT and 95% CI are shown with overlaid jittered data points at each visit. Plot will have only one panel that shows all four study groups.

Figure 4: **Serologic IgG Responses to *S. flexneri* 2a Invaplex (Visit 01, 04, 07, 09, 10, 11): Distribution of Titers Using Reverse Cumulative Distribution Curves - Immunogenicity Population**

Figure 5: **Serologic IgG Responses to *S. flexneri* 2a Invaplex (Visit 01, 04, 07, 09, 10, 11): Geometric Mean Titer and 95% CI - Immunogenicity Population**

Figure 6: **Antibody in Lymphocyte Supernatant (ALS) IgA Responses to *S. flexneri* 2a Invaplex (Visit 00B, 01, 03, 06, 09, 11): Distribution of Titers Using Reverse Cumulative Distribution Curves - Immunogenicity Population**

Figure 7: **Antibody in Lymphocyte Supernatant (ALS) IgA Responses to *S. flexneri* 2a Invaplex (Visit 00B, 01, 03, 06, 09, 11): Geometric Mean Titer and 95% CI - Immunogenicity Population**

Figure 8: **Antibody in Lymphocyte Supernatant (ALS) IgG Responses to *S. flexneri* 2a Invaplex (Visit 00B, 01, 03, 06, 09, 11): Distribution of Titers Using Reverse Distribution Curves - Immunogenicity Population**

Figure 9: **Antibody in Lymphocyte Supernatant (ALS) IgG Responses to *S. flexneri* 2a Invaplex (Visit 00B, 01, 03, 06, 09, 11): Geometric Mean Titer and 95% CI - Immunogenicity Population**

APPENDIX C. LISTINGS

LIST OF LISTINGS

C.1 Demographics Listings

Listing 1: Discontinued Subjects

Treatment Group	Subject ID	Category	Reason for Early Termination or Treatment Discontinuation	Study Visit

Listing 2: Subject Specific Protocol Deviation

Treatment Group	Subject ID	DV Number	Deviation	Deviation Category	Study Visit	Reason for Deviation	Deviation Resulted in AE?	Deviation Resulted in Subject Termination?	Deviation Affected Product Stability?	Deviation Resolution	Excluded from PPP	Major/ Minor
Comment:												

Listing 3: Non-Subject-Specific Protocol Deviations

Start Date	Deviation	End Date	Reason for Deviation	Deviation Resulted in Subject Termination?	Deviation Affected Product Stability?	Deviation Category	Deviation Resolution	Comments

Listing 4: Demographics

Treatment Group	Subject ID	Sex	Age at Enrollment (years)	Ethnicity	Race

Listing 5: Pre-Existing Conditions

Treatment Group	Subject ID	MH Number	Medical History Term	Condition Start Day	Condition End Day	MedDRA System Organ Class	MedDRA Preferred Term

C.2 Immunogenicity Listings

Listing 6: Serum IgA Antibody against *S. flexneri 2a* Invaplex

		Baseline	Visit 04		Visit 07		Visit 09		Visit 10		Visit 11	
Study Group	Subject ID	Titer	Titer	Fold Rise	Titer	Fold Rise	Titer	Fold Rise	Titer	Fold Rise	Titer	Fold Rise

Listing 7: Serum IgG Antibody against *S. flexneri 2a* Invaplex

		Baseline	Visit 04		Visit 07		Visit 09		Visit 10		Visit 11	
Study Group	Subject ID	Titer	Titer	Fold Rise	Titer	Fold Rise	Titer	Fold Rise	Titer	Fold Rise	Titer	Fold Rise

Listing 8: ALS IgA Antibody against *S. flexneri 2a* Invaplex

		Day -7	Baseline	Visit 03		Visit 06		Visit 09		Visit 11	
Study Group	Subject ID	Titer	Titer	Titer	Fold Rise	Titer	Fold Rise	Titer	Fold Rise	Titer	Fold Rise

Listing 9: ALS IgG Antibody against *S. flexneri 2a* Invaplex

		Day -7	Baseline	Visit 03		Visit 06		Visit 09		Visit 11	
Study Group	Subject ID	Titer	Titer	Titer	Fold Rise	Titer	Fold Rise	Titer	Fold Rise	Titer	Fold Rise

C.3 Safety Listings

Listing 10: Adverse Events, by MedDRA System Organ Class (SOC) and Preferred Term (PT)

[SOC1], [PT1]

Treatment Group	Subject ID	Adverse Event	Dose Number	Onset Day Post-Dose (Duration)	Severity	SAE	Relationship to Study Treatment	In Not Related, Alternative Etiology	Action Taken with Study Treatment	Subject Discontinued Due to AE?	Outcome
						Y/N				Y/N	
AE Summary/Comment:											

Listing 11: Clinical Lab Values – Hematology

Time Point [1]	Actual Study Day	Reference Range	Value	Change from Baseline	Severity	Clinically Significant	Comment
Subject ID: XXXXXX, Treatment Group: YYYYYY							
[Parameter 1]							
Screening							
Day -7							
Day 8							
Day 29							
Day 50							
[Parameter 2]							
Screening							
Day -7							
Day 8							
Day 29							
Day 50							
[continue for other parameters]							

Listing 12: Clinical Lab Values – Chemistry

Similar format to Listing 11.

Listing 13: Vital Signs

Treatment Group	Subject ID	Time Point	Actual Study Day	Temperature (°C)	Systolic Blood Pressure (mmHg)	Diastolic Blood Pressure (mmHg)	Heart Rate (beats/min)	Weight (kg)	Height (cm)

Listing 14: Concomitant Medications

Study Group	Subject ID	CM Number	Medication	Medication Start Day	Medication End Day	Ongoing at Study Start	Indication	Taken for an AE? (AE Description; Number)	Taken for a condition on Medical History? (MH Description; Number)	ATC Level 1 (ATC Level 2)

Listing 15: Pregnancy Report

Subject ID: XXXXX, Treatment Group: YYYYYY

Maternal Information	
Pregnancy Number	
Date pregnancy reported	
Pregnancy test date	
Pregnancy status	
Estimated date of delivery	
Number of fetuses	
Actual date of delivery	
Maternal complications	
Action Taken with Study Product	
Study Status	
Comments	
Gravida and Para	
Gravida	
Pre-Term Birth	Extremely / Very early / Early / Late
Term Birth	Early / Full / Late / Post
Still Birth	
Abortion	Spontaneous / Miscarriage / Elective / Therapeutic
Major Congenital Anomaly with Previous Pregnancy?	
Live / Still Birth Outcomes (include only fields relevant to outcome)	
Fetus Number	
Pregnancy outcome	
Fetal Distress During Labor and Delivery?	
Delivery method	
Gestational Age at Live/Still Birth	
Size for Gestational Age	
Apgar Score, 1 minute	
Apgar Score, 5 minutes	
Cord pH	
Congenital anomalies	
Autopsy performed	
If Autopsy, Etiology for Still Birth Identified?	
Illnesses/ Hospitalizations within 1 Month of Birth?	
Spontaneous, Elective, or Therapeutic Abortion Outcomes	
Fetus Number	
Date of Initial Report	
Pregnancy Outcome	
Gestational Age at termination	
Abnormality in Product of Conception?	
Reason for Therapeutic Abortion	

[Only include one of the last 2 sections, where appropriate: i.e., Live / Still birth or abortion.]