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


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## Investigator's Agreement

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

*"I have read this protocol and agree to conduct the study as outlined herein in accordance with International Council on Harmonisation Good Clinical Practice Guideline and FDA, DoD, and United States Army Regulations."*

GUTIERREZ.RAMIRO.LUIS.1187040856 Digitally signed by GUTIERREZ.RAMIRO.LUIS.1187040856 Date: 2019.05.22 15:06:25 -04'00'

22MAY2019

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EDD, NMRC

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## 2. Synopsis

| | | |
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| **Investigational Product** | Detoxified *Shigella flexneri* 2a Artificial Invaplex (Invaplex$_{AR\text{-}DETOX}$) (Lot 1972) | |
| **Short Protocol Title** | Safety and immunogenicity of Detoxified Artificial Invaplex (*Shigella flexneri* 2a Invaplex$_{AR\text{-}DETOX}$) | |
| | Clinical Phase: 1 | IND Number: 18707 |
| **Sponsor** | PATH | |
| **Vaccine Manufacturer** | Walter Reed Army Institute of Research (WRAIR) Pilot BioProduction Facility (PBF) | |
| **Study Sites** | WRAIR Clinical Trials Center (CTC) | |
| **Principal Investigator** | Ramiro Gutierrez, MD, MPH | |
| **Study Duration** | Screening (up to 60 days); vaccination to final blood draw (70 days); telephone follow-up: 6 months after final dose; immunology analysis (12 weeks); data analysis and report writing (2 months). Advancement between cohorts will be reviewed by the Protocol Safety Review Team (PSRT) and occur upon review of safety data through 7 days after the 3$^{rd}$ dose (approximately 2-4 weeks between cohorts). The clinical phase of the study will last for approximately 1 year followed by another year of data analysis and report writing. | |
| **Study Objectives** | **Primary:** Evaluate the safety of detoxified *Shigella flexneri* 2a Invaplex$_{AR}$ (Invaplex$_{AR\text{-}DETOX}$) administered by intramuscular (IM) immunization.<br>**Secondary:** Evaluate immune responses (serum IgG/IgA and ALS IgG/IgA) following IM immunization with Invaplex$_{AR\text{-}DETOX}$.<br>**Exploratory:**<br>• Collect samples from vaccinated subjects for exploratory immunological assays.<br>• Perform secondary immunology analyses.<br>• Collect samples for evaluation of C-reactive protein (CRP) responses to the vaccine and placebo. | |

<table>
<tr><td>Study Design</td><td>This is a randomized, double-blind, placebo-controlled, Phase 1 clinical trial in which a total of 60 subjects will receive one of three doses of Invaplex$_{AR\text{-}DETOX}$ or placebo (saline), as shown in the table below. The vaccine will be administered via IM injection on study days 1, 22, and 43. Each subject will receive the same formulation at each vaccination dependent upon group assignment.<br>The study will be initiated with the lowest dose level (2.5 µg) and will proceed to the next highest dose 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 higher level. All safety data will be summarized and reviewed by the PSRT prior to dose-escalation.<br>For the initial cohort, enrollment will be limited initially to 4 subjects receiving 2.5 µg of Invaplex$_{AR\text{-}DETOX}$ alone and 1 subject receiving placebo. If no unexpected symptoms occur within 7 days following initial vaccination, enrollment of the remaining subjects in the cohort may proceed.
<table>
<tr><th>Cohort</th><th>Group</th><th>n</th><th>Route</th><th>Invaplex$_{AR\text{-}DETOX}$ (µg)</th></tr>
<tr><td rowspan="2">A</td><td>A-1</td><td>16</td><td>IM</td><td>2.5</td></tr>
<tr><td>A-2</td><td>4</td><td>IM</td><td>Placebo</td></tr>
<tr><td rowspan="2">B</td><td>B-1</td><td>16</td><td>IM</td><td>10</td></tr>
<tr><td>B-2</td><td>4</td><td>IM</td><td>Placebo</td></tr>
<tr><td rowspan="2">C</td><td>C-1</td><td>16</td><td>IM</td><td>25</td></tr>
<tr><td>C-2</td><td>4</td><td>IM</td><td>Placebo</td></tr>
</table>
Specimens will be collected at prescribed intervals to examine systemic and mucosal immune responses. Vaccine safety will be actively monitored during vaccination and for 28 days following the third vaccine dose. Additionally, a telephone follow-up will be performed at 6 months following receipt of the final vaccination to assess for adverse events.</td></tr>
<tr><td>Primary Clinical Outcome</td><td>The primary clinical outcome for this study is the assessment of safety and tolerability of Invaplex$_{AR\text{-}DETOX}$. All subjects receiving investigational product will be included in the safety assessment. For safety laboratory data, including hematology, serology, and chemistry parameters, blood will be collected at screening and study days as per the Time and Events Schedule (Table 8). Post-vaccination safety monitoring will include laboratory and clinical evaluations following each vaccine/placebo administration, including assessment of post-vaccination local and systemic reactions through targeted physical exams, symptom surveys, symptom diaries, and other adverse event (AE) monitoring.</td></tr>
<tr><td>Immunology Outcomes</td><td>Primary Immunology Outcomes: The primary immunological outcomes will be Antibodies in Lymphocyte Supernatant (ALS) and antibody (IgG and IgA) titers against Invaplex using methods previously established. Seroconversion will be defined as a ≥ 4-fold increase in endpoint titer between pre-and post-vaccination samples. Peripheral Blood Mononuclear Cells (PBMCs) will be collected to determine antigen-specific IgG and IgA titers in ALS samples. High-titer specimens will be included on each plate to track day to day inter-assay variation. For each antigen, pre- and post-vaccination serum samples will be assayed side-by-side. The antibody titer assigned to each sample will represent the geometric mean of duplicate tests. Reciprocal endpoint titers less than the starting dilution of the assay will be assigned a value of half the starting dilution for computational purposes.<br><br>Secondary and Exploratory Evaluations: Serologic and ALS (IgG and IgA) responses to additional antigens (S. flexneri 2a LPS, IpaB, IpaC) may be assessed depending on sample availability. IgM and IgG subclass responses (serum and ALS) to key antigens may also be assessed. Serum bactericidal (SBA) titers against S. flexneri 2a 2457T will be determined for pre- and post-vaccination samples. An SBA responder will be defined as ≥ 9-fold increase in SBA titer as</td></tr>
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| | compared to baseline SBA titers. Samples will be collected, processed, and archived for analysis. PBMCs will be collected for antigen-specific memory B and T cell analysis, antibody secreting cell assay and α4β7+ B cells. Stool samples will be collected to assess antigen-specific fecal IgA and IgG responses. A fecal response will be defined as a ≥ 4-fold increase from baseline. Concurrently, saliva samples may be collected to assess mucosal IgA and IgG antibody responses. |
| **Hypotheses** | **Safety:** One or more of the proposed dosing regimens will have an acceptable frequency and severity of adverse events.<br><br>**Immunogenicity:**<br>• IM administration of Invaplex$_{AR\text{-}DETOX}$ will induce serum IgG antibody responses to *S. flexneri* 2a Invaplex in ≥50% of subjects in at least one of the study groups.<br>• IM administration of Invaplex$_{AR\text{-}DETOX}$ will induce antibody responses to either IpaB or IpaC in ≥50% of subjects in at least one of the study groups. |
| **Estimated Number of Subjects Screened** | Based on prior studies with similar study designs, sample collections and inclusion/exclusion criteria, we anticipate screening approximately 5 subjects for every one subject that is enrolled in the study. Therefore we anticipate approximately 300 individuals will be screened. |
| **Maximum Number of Subjects Enrolled** | A maximum of 60 subjects are planned for this study. Up to four alternates per group will be selected that may be enrolled in the event a subject becomes ineligible prior to receipt of the investigational product. Subjects receiving at least one dose of the vaccine (or placebo) will not be replaced. |
| **Eligibility Criteria** | **Inclusion Criteria:**<br>• Healthy, adult, male or female, age 18 to 50 years (inclusive) at the time of enrollment.<br>• Completion and review of comprehension test (achieved ≥ 70% accuracy, two attempts allowed).<br>• Provide written informed consent before initiation of any study procedures.<br>• Agrees to complete all study visits and procedures and to provide a screening stool sample.<br>• 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.<br>**Exclusion Criteria:**<br>*General health*<br>• Health problems (for example, chronic medical conditions such as psychiatric conditions, diabetes mellitus, hypertension, or any other conditions that might place the subjects 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.<br>• History of autoimmune disorders, cardiovascular and renal disease.<br>• Use of immunosuppressive medications (systemic corticosteroids or chemotherapeutics that may influence antibody development), or immunosuppressive illness, including IgA deficiency (defined by serum IgA <7mg/dL).<br>• 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. |

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| | • Participation in research involving another investigational product (defined as receipt of an investigational product or exposure to an invasive investigational device) 30 days before planned date of first vaccination or anytime throughout the duration of the study until the last in-clinic study safety visit.<br>• Positive blood test for HBsAG, HCV Ab, HIV-1/HIV-2 Ab.<br>• Clinically significant abnormalities on basic laboratory screening tests.<br>• Systemic antimicrobial treatment (i.e., topical treatments are not an exclusion) within 1 week before administration of the first vaccine dose.<br>*Research specific*<br>• Allergies that may increase the risk of AEs.<br>• Regular use (weekly or more often) of antidiarrheal, anti-constipation, or antacid therapy.<br>• 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.<br>• Personal or family history of an inflammatory arthritis.<br>• Positive blood test for HLA-B27 (associated with increased risk of reactive arthritis secondary to *Shigella* infection)<br>• History of allergy to any vaccine.<br>• Exclusionary skin disease history/findings that would confound assessment or prevent appropriate local monitoring of AEs, or possibly increase the risk of a local AE.<br>*Prior Exposure to Shigella*<br>• Serum IgG titer > 2500 to *Shigella flexneri* 2a LPS.<br>• History of microbiologically confirmed *Shigella* infection.<br>• Received previous licensed or experimental *Shigella* vaccine or live *Shigella* challenge.<br>• Travel to countries where *Shigella* or other enteric infections are endemic (most of the developing world) within two years prior to dosing (clinician judgement).<br>• Occupation involving handling of *Shigella* bacteria currently, or in the past 3 years. |
| **Sample Size** | The sample size of this study is limited by the early stage (Phase 1) of the product concept/testing and is 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 our estimate for adverse events is limited. For example, using binomial probability formula for no observed adverse events within the 16 subjects per group yields a 95% confidence interval of 0-21%. Follow-on studies evaluating seemingly safe and immunogenic doses will be required with larger numbers of volunteers in order to better define the safety profile. |
| **Statistical Methods** | 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. Summary tables will be created to indicate the number of subjects who experience events. Vaccine-related events (definitely, probably, or possibly related) will be tabulated by study group. In addition, tables will be prepared to list each adverse event, the number of subjects in each treatment group who experienced an event at least once, and the rate of subjects with adverse event(s). Adverse events will be divided into defined severity grades (mild, moderate, severe, potentially life-threatening). The tables will also |

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| | divide the adverse events by relationship to the investigational product if applicable. All immunized subjects will be included in the safety analysis.<br><br>For immunology responses, both qualitative (responder rates) and quantitative (e.g., $log_{10}$ transformed values) results will be analyzed. Geometric mean titers will be calculated for serologic and ALS responses along with their 95% confidence intervals (or standard deviation). If appropriate, between groups comparisons will be examined with nonparametric median tests (Kruskal-Wallis for continuous data and Fisher's exact tests for categorical data) unless assumptions are fulfilled for Student's t or $\chi^2$.<br><br>Additional comparisons may be made using repeated measures analysis of variance with study group as the between subject factor and sample collection time-points as the repeated factor. All statistical tests will be interpreted in a two-tailed fashion using an alpha = 0.05 to represent statistical significance. |

## 3. Table of Contents, List of Tables and List of Figures

### Table of Contents

## List of Tables



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## 4. List of Abbreviations and Definitions of Terms

**Table 2: Abbreviations**

| Abbreviation | Explanation |
|---|---|
| µg | Microgram(s) |
| µL | Microliter(s) |
| AE | Adverse event, adverse experience |
| ALS | Antibody lymphocyte supernatant |
| ASC | Antibody-secreting cell |
| C | Celsius |
| CCHMC | Cincinnati Children's Hospital Medical Center |
| CFR | Code of Federal Regulations |
| cfu | Colony forming units |
| cGMP | Current good manufacturing practice(s) |
| CHNRI | Child Health and Nutrition Research Initiative |
| CRP | C-Reactive Protein |
| CTC | Clinical Trials Center |
| dmLT | Double mutant heat labile toxin |
| DoD | Department of Defense |
| eCRF | Electronic case report form |
| EDC | Electronic data capture |
| ETEC | Enterotoxigenic *Escherichia* coli |
| F | Fahrenheit |
| FDA | US Food and Drug Administration |
| GCP | Good clinical practice(s) |
| GLP | Good laboratory practice(s) |
| GMP | Good manufacturing practice(s) |
| HBsAG | Hepatitis B surface antigen |
| HCV | Hepatitis C virus |
| HIV | Human immunodeficiency virus |
| HRPO | Human Research Protections Office |
| IBS | Irritable bowel syndrome |
| ICH | International Council on Harmonisation |
| ID | Intradermal |
| IgA | Immunoglobulin A |
| IgG | Immunoglobulin G |
| IgM | Immunoglobulin M |
| IM | Intramuscular(ly) |
| IN | Intranasal(ly) |
| IND | Investigational New Drug |
| Invaplex$_{AR}$ | Artificial Invaplex |
| Invaplex$_{AR\text{-}DETOX}$ | Detoxified Artificial Invaplex |
| Invaplex$_{NAT}$ | Native Invaplex |
| IRB | Institutional Review Board |

| Abbreviation | Explanation |
|---|---|
| LPS | Lipopolysaccharide antigen |
| mL | Milliliter(s) |
| ml | Milliliters |
| MOP | Manual of Procedures |
| NMRC | Naval Medical Research Center |
| PBF | Pilot BioProduction Facility |
| PBMC | Peripheral Blood Mononuclear Cells |
| PI | Principal investigator |
| PSRT | Protocol Safety Review Team |
| PVS | PATH Vaccine Solutions |
| SAE | Serious adverse event |
| SAP | Statistical analysis plan |
| SBA | Serum bactericidal activity |
| SDS-PAGE | Sodium dodecyl sulfate – polyacrylamide gel electrophoresis |
| SEC-HPLC | Size exclusion chromatography – high pressure liquid chromatography |
| SOP | Standard operating procedures |
| SOS | Salimetrics Oral Swab |
| T3SS | Type-III Secretion System |
| TD | Travelers' diarrhea |
| USAMRMC | US Army Medical Research and Materiel Command |
| WIRB | Western Institutional Review Board |
| WRAIR | Walter Reed Army Institute of Research |
| WRNMMC | Walter Reed National Military Medical Center |

## 5. Introduction

There are approximately 4.6 billion global annual diarrheal cases [1]. Diarrhea is a leading cause of mortality in children under 5 years and negatively impacts growth and cognitive development. Furthermore, bacillary diarrhea is a continuing problem for American military personnel and civilian travelers regions where *Shigella* is endemic.

Shigellosis or bacillary dysentery is a food- and waterborne disease predominantly implicated in children < 5 years of age. Estimates of *Shigella*-associated morbidity and mortality range from 0.1 to 1.1 million deaths (60% in children under 5) and from 90 to 165 million cases of dysentery annually [2, 3]. In military populations, *Shigella* species are a common cause of travelers' diarrhea (TD) responsible for approximately 7% of TD cases [4]. In addition to the acute morbidity of diarrhea in this population, shigellosis is linked to several post-infectious sequelae including irritable bowel syndrome (IBS) and reactive arthritis [5-7].

As *Shigella* species invade the intestinal mucosa, the disease is less amenable to the salutary effects of oral rehydration compared to enterotoxigenic pathogens that cause dehydrating diarrhea [8]. Therefore, antibiotics are frequently required for treatment; however, increasing resistance to fluoroquinolones and cephalosporins (extended spectrum β-lactamases) is undermining efficacy. As therapeutic options narrow, the need for a safe and effective *Shigella* vaccine becomes more pressing. An expert panel convened by the Child Health and Nutrition Research Initiative (CHNRI) of the World Bank identified *Shigella* as one of the highest priorities for long-term vaccine development [2]. Furthermore, the Department of Defense (DoD) has issued directives for the development of vaccines against Campylobacter, enterotoxigenic *Escherichia coli* (ETEC), and *Shigella* (DoD Directive 6205.3; BUMEDINSTR 5450.171).

There are 4 different species [and 47 antigenically distinct serotypes of *Shigella*, divided on the basis of differences in their outer membrane lipopolysaccharide antigen (LPS)], which are *Shigella dysenteriae* (13 serotypes), *S. flexneri* (16 serotypes), *S. boydii* (18 serotypes), and *S. sonnei* (1 serotype) [2]. The multiple and evolving serotypes require the development of polyvalent vaccines [9]. Recent vaccine development efforts have targeted *S. flexneri* 2a, 3a, and 6 as well as *S. sonnei* in an effort to provide broad coverage against the most predominant *Shigella* strains.

*Shigella* pathogenesis and immunity are attributed to the organism's ability to invade, replicate intra-cellularly, and spread inter- and intra-cellularly within the colonic epithelium [8]. This invasion eventually results in extensive inflammation and tissue destruction of the villous epithelium adjacent to lymphoid follicles resulting in the formation of mucosal micro-abscesses and ulcerations [8]. As contiguous epithelial cells die, additional areas of mucosal ulceration and hemorrhage are formed.

To initiate infection, *Shigella* utilize a Type-III Secretion System (T3SS). The T3SS consists of proteins that comprise the needle complex (> 12 proteins) and nonstructural secreted proteins forming a conduit for the passage of virulence proteins from the pathogen to the enterocyte. The needle apparatus consists of a membrane-embedded basal anchor-like structure, an external needle that protrudes from the bacterial surface (which allows passage of effector proteins), and a tip complex that caps the needle and functions to sense the presence of eukaryotic cells (also serving as a platform of the translocon and regulating the effector protein secretion). Upon host

cell contact, a translocon is assembled between the needle tip complex and the host cell, serving as a gateway for translocation of effector proteins by creating a pore in the host cell membrane. IpaD, located at the needle tip senses the environment and the location in the gastrointestinal tract, recruiting IpaB to the needle tip upon host cell recognition. The needle complex then interacts with the host cell and inserts itself to deliver *Shigella* effector proteins. Because the Ipa proteins are conserved across all *Shigella* spp. and are directly responsible for initiating infection, immune defenses against the TTSS effector proteins may confer broad-spectrum protection against multiple serotypes overcoming the homologous protection afforded by an LPS only strategy [10].

Immunization strategies for *Shigella* vaccines to date have included killed whole-cell bacteria, live-attenuated bacteria, recombinant carrier organisms, polysaccharide conjugates, and LPS-protein mixtures. These approaches have often proven either too reactogenic or poorly immunogenic in human testing. Oral vaccines may activate multiple effector arms of the immune system; however, they are difficult to administer in doses that are immunogenic, safe, and well tolerated and suffer from delivery restrictions [erratic absorption, degradation in acidic environment], particularly for inactivated or subunit vaccines compromising reproducible responses. Development of an immunogenic, safe intramuscular (IM) *Shigella* vaccine is crucial to harmonize immunization with other vaccines administered globally, particularly as use of alternate routes would necessitate specialized training for administering personnel.

## 5.1. Invaplex$_{NAT}$

The *Shigella* invasin complex (termed, Invaplex) containing IpaB, IpaC and LPS underwent manufacturing and initial preclinical and clinical testing in the early 2000s. That formulation, termed *S. flexneri* 2a native Invaplex or *S. flexneri* 2a Invaplex$_{NAT}$ was a macromolecular complex isolated from wild-type *S. flexneri* 2a via FPLC Ion-exchange chromatography purification. In addition to IpaB, IpaC, and LPS, this formulation contained other invasins (IpaA, IpaD), the VirG protein, two non-virulence specific proteins of 72 and 84 kDa molecular weight (DnaK and elongation factor-G, respectively) and several other proteins of various molecular weights and limited immunogenicity.

*S. flexneri* 2a Invaplex$_{NAT}$ was safely administered intranasally (IN) in small animals and stimulated robust mucosal and serum intestinal and pulmonary immune responses [humoral, Th1 and Th2 directed at IpaB, IpaC, and LPS and intestinal IgA responses] [11, 12]. The *S. flexneri* 2a Invaplex$_{NAT}$ vaccine was protective in guinea pigs using the keratoconjunctivitis model and in mice using the lethal pneumonia model against both *S. sonnei* and *S. flexneri* [11, 13-15]. The optimal immunization schedule in mice (5 µg per dose) and guinea pigs (25 µg per dose) was 3 doses given at 2-week intervals. Immunological memory was observed in mice 15 weeks after the final immunization.

### 5.1.1. Synopsis: Human *S. flexneri* 2a Invaplex IN Investigations

Two lots of *S. flexneri* 2a Invaplex$_{NAT}$ were administered to 117 human subjects in 3 investigations at doses ranging from 10 to 690 µg [12, 16]. The first clinical trial of *S. flexneri* 2a Invaplex$_{NAT}$ was a dose-escalation study conducted using doses ranging from 10 to 480 µg with an initial lot of *S. flexneri* 2a Invaplex$_{NAT}$ (Lot 0994) [12, 16]. A total of 3 doses (2 weeks apart) of vaccine were administered IN to each subject using a pipette. The most commonly reported

symptoms were rhinorrhea and nasal congestion seen in 50% and 47%, respectively. Mild nasal discharge and nasal turbinate edema were observed on clinical exam in 63% and 50% of volunteers. There were no significant differences in the frequency of these adverse events across dose levels or by dose. The majority (99%) of symptoms/signs were mild. High pre-vaccination findings were also observed (nasal discharge: 46%; nasal edema: 38%; rhinorrhea: 21%; and nasal congestion: 17%). Safety laboratory tests showed no significant changes from baseline among any of the study groups. Doses ≥ 240 µg induced increasing frequency of immune responses (Table 3); however, the magnitude of immune responses was moderate.

**Table 3: Frequency (%) of Immune Responses to Immunizing Antigen(s) Following First in Human Phase 1 Clinical Trial of *S. flexneri* 2a Invaplex$_{NAT}$ (Lot 0994)**

| Antigen | Assay | Study Group | | | |
|---|---|---|---|---|---|
| | | A (n = 7) | B (n = 8) | C (n = 7) | D (n = 8) |
| | | 10 µg | 50 µg | 240 µg | 480 µg |
| *S. flexneri* 2a Invaplex$_{NAT}$ | IgA ASC | 0.0 | 25.0 | 42.9 | 25.0 |
| | IgG ASC | 0.0 | 25.0 | 100 | 50.0 |
| | Serum IgA | 0.0 | 12.5 | 14.3 | 0.0 |
| | Serum IgG | 0.0 | 0.0 | 14.3 | 0.0 |
| | Fecal IgA | 0.0 | 12.5 | 28.6 | 37.5 |
| LPS | IgA ASC | 0.0 | 12.5 | 42.9 | 25.0 |
| | IgG ASC | 0.0 | 12.5 | 71.4 | 37.5 |
| | Serum IgA | 0.0 | 12.5 | 0.0 | 0.0 |
| | Serum IgG | 0.0 | 0.0 | 28.6 | 0.0 |
| | Fecal IgA | 0.0 | 12.5 | 28.6 | 50.0 |

Note: Analysis limited to subjects receiving at least 2 doses of *S. flexneri* 2a InvaplexNAT and having post-Dose 2 blood samples collected.

The results of this study led to a second in human clinical investigation, a randomized, double-blind, placebo-controlled study with four groups [3 groups with 12 subjects each were randomized to 1 of 3 *S. flexneri* 2a Invaplex$_{NAT}$ dose levels—240 µg, 480 µg, or 690 µg]. The test article was administered with the Dolphin™ nasal delivery device [purported to optimize IN delivery via nasal mucosal deposition subsequently improving the immune response]. Additionally, a cohort of 8 subjects received 240 µg of the new vaccine Lot 1307 via pipette as a bridge to the first in human clinical trial [to compare the relative superiority in the delivery devices (Dolphin™ to pipette)]. The 690 µg dose of *S. flexneri* 2a Invaplex$_{NAT}$ (Lot 1307) was selected based on data from the phase 1 clinical trial. Similar to the first-in-human clinical trial, the most commonly reported symptoms were rhinorrhea (31%) and nasal congestion (33%). Sneezing and nasal itching were also observed relatively frequently (both seen in 28% of the subjects). The majority (98%) of all vaccine-associated adverse events were classified as mild. No subjects reported any related severe or serious adverse events. Table 4 shows the frequency of individual immune responses to the immunizing antigens (*S. flexneri* 2a Invaplex$_{NAT}$, LPS).

Serologic responses were relatively uncommon with no more than 20% of the vaccine recipients exhibiting a ≥ 4-fold rise over baseline titers to either of the 2 studied antigens (*S. flexneri* 2a

Invaplex$_{NAT}$ and LPS). Antibody-secreting cell (ASC) responses were more common and seen in 50%-60% of the vaccine recipients depending on the antigen evaluated. Positive antigen-specific ASC responses were detected in volunteers from all study groups. In general, antigen-specific IgA and IgG ASCs peaked following the second vaccination in the majority (75%–77%) of volunteers regardless of vaccine dose (data not shown). The 690 µg dose elicited the greatest response rate (maximum with IgA ASC—75%) and magnitude of response. Of importance, vaccination with comparable doses of the *S. flexneri* 2a Invaplex$_{NAT}$ LPS/protein subunit complex via the Dolphin™ resulted in higher plasma and ASC immune responses as compared to pipette delivery confirming the importance of optimizing IN deposition in eliciting optimal immune responses.

**Table 4: Frequency (%) of Immune Responses to Immunizing Antigen(s) Following Second Phase 1 Clinical Trial of *S. flexneri* 2a Invaplex$_{NAT}$ Lot 1307**

| | ***S. flexneri* 2a Invaplex$_{NAT}$** | | | | **LPS** | | | |
|---|---|---|---|---|---|---|---|---|
| | **Serology** | | **ASC** | | **Serology** | | **ASC** | |
| **Group** | **IgA** | **IgG** | **IgA** | **IgG** | **IgA** | **IgG** | **IgA** | **IgG** |
| **A (240 µg) n = 11** | 0.0 | 27.3 | 18.2 | 27.3 | 9.1 | 45.5 | 27.3 | 36.4 |
| **B (480 µg) n = 10** | 10.0 | 30.0 | 20.0 | 20.0 | 10.0 | 40.0 | 20.0 | 20.0 |
| **C (690 µg) n = 12** | 50.0 | 16.7 | 75.0 | 58.3 | 25.0 | 41.7 | 58.3 | 58.3 |
| **D (240 µg) n = 8** | 0.0 | 0.0 | 14.3 | 14.3 | 0.0 | 0.0 | 14.3 | 14.3 |

Note: Analysis limited to subjects receiving at least 2 doses of *S. flexneri* 2a native Invaplex$_{NAT}$ and having post-Dose 2 blood samples collected.
Groups A, B, and C were immunized with nasal delivery device; group D was immunized with a micropipette

In a follow-on double-blind, placebo-controlled, vaccination-challenge study, a total of 52 subjects were randomized to receive 690 µg of *S. flexneri* 2a Invaplex$_{NAT}$ (n=31) or placebo (n = 21). Other than sneezing, which was more common in vaccine recipients (Chi-square p = 0.03), there were no significant differences in the frequency of adverse events between the 2 study groups. Additionally, the majority of all adverse events were of mild severity and no adverse events were coded as severe. Further, there were no adverse events that met the vaccination stopping criteria, nor were there any vaccine-related serious adverse events. Based on a priori subject selection criteria, 10 vaccinees and 12 placebo recipients advanced to the challenge phase of the study. All 22 subjects were admitted and challenged with 800 cfu of live *S. flexneri* 2a strain 2457T. There were no significant differences in the rate of fever, diarrhea, or any of the diarrhea severity indices between vaccine recipients selected to advance to challenge and placebo recipients.

Throughout the course of several clinical trials with *S. flexneri* 2a Invaplex$_{NAT}$, there were no adverse events that met the vaccination stopping criteria, nor were there any vaccine-related

serious adverse events. Table 5 details the vaccine-related, surveyed signs and symptoms of subjects across the 2 lots and the range of doses. The most common adverse symptoms include nasal congestion (33.3%), rhinorrhea (29.9%), and sneezing (25.6%), all of mild severity. Placebo recipients have experienced similar rates of solicited symptoms. The only solicited local physical exam finding or solicited symptom that appeared to be significantly different between vaccinees and placebo recipients (18.8% and 0.0%, respectively; $p = 0.03$) was mild nasal itching; however, there was no significant increase in the rate of nasal itching with increasing vaccine doses (chi-square test for trend $p = 0.70$).

## Table 5: Number (%) of Subjects with Vaccine Related Solicited Signs and Symptoms

| Lot Number | 0994 | 0994 | 0994 | 0994 | 1307 | 1307 | 1307 | 1307 | Placebo |
|---|---|---|---|---|---|---|---|---|---|
| Delivery Device | Pipet | Pipet | Pipet | Pipet | Pipet | Dolphin™ | Dolphin™ | Dolphin™ | Dolphin™ |
| Dose | 10 µg | 50 µg | 240 µg | 480 µg | 240 µg | 240 µg | 480 µg | 690 µg | -- |
| N | 8 | 8 | 8 | 8 | 8 | 12 | 12 | 53[a] | 28[b] |
| Malaise | 0 (0.0) | 1 (12.5) | 2 (25.0) | 0 (0.0) | 1 (12.5) | 1 (8.3) | 0 (0.0) | 6 (11.3) | 2 (7.1) |
| Headache | 0 (0.0) | 4 (50.0) | 2 (25.0) | 4 (50.0) | 1 (12.5) | 1 (8.3) | 1 (8.3) | 8 (15.1) | 7 (25.0) |
| Rhinorrhea | 2 (25.0) | 4 (50.0) | 5 (62.5) | 5 (62.5) | 1 (12.5) | 4 (33.3) | 3 (25.0) | 11 (20.8) | 5 (17.9) |
| Nasal congestion | 3 (37.5) | 3 (37.5) | 3 (37.5) | 6 (75.0) | 2 (25.0) | 7 (58.3) | 2 (16.7) | 13 (24.5) | 7 (25.0) |
| Nasal burning | 0 (0.0) | 2 (25.0) | 2 (25.0) | 1 (12.5) | 1 (12.5) | 1 (8.3) | 0 (0.0) | 8 (15.1) | 2 (7.1) |
| Nasal itching | 1 (12.5) | 2 (25.0) | 1 (12.5) | 2 (25.0) | 1 (12.5) | 1 (8.3) | 4 (33.3) | 10 (18.9) | 0 (0.0) |
| Sore throat | 2 (25.0) | 0 (0.0) | 1 (12.5) | 4 (50.0) | 1 (12.5) | 2 (16.7) | 0 (0.0) | 10 (18.9) | 4 (14.3) |
| Postnasal drip | 2 (25.0) | 0 (0.0) | 5 (62.5) | 3 (37.5) | 2 (25.0) | 2 (16.7) | 2 (16.7) | 10 (18.9) | 5 (17.9) |
| Cough | 0 (0.0) | 2 (25.0) | 1 (12.5) | 1 (12.5) | 0 (0.0) | 1 (8.3) | 0 (0.0) | 8 (15.1) | 1 (3.6) |
| Sinus pain | 0 (0.0) | 0 (0.0) | 1 (12.5) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 4 (7.5) | 1 (3.6) |
| Sneezing | 2 (25.0) | 2 (25.0) | 3 (37.5) | 3 (37.5) | 0 (0.0) | 1 (8.3) | 4 (33.3) | 15 (28.3) | 4 (14.3) |
| Itching eyes | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (25.0) | 0 (0.0) | 1 (8.3) | 1 (8.3) | 5 (9.4) | 2 (7.1) |
| Nose bleed | 0 (0.0) | 0 (0.0) | 0 (0.0) | 2 (25.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 4 (7.5) | 0 (0.0) |
| Nasal mucosa hyperemia | 2 (25.0) | 5 (62.5) | 0 (0.0) | 1 (12.5) | 1 (12.5) | 1 (8.3) | 0 (0.0) | 15 (28.3) | 7 (25.0) |
| Nasal discharge | 3 (37.5) | 7 (87.5) | 5 (62.5) | 5 (62.5) | 1 (12.5) | 1 (8.3) | 0 (0.0) | 6 (11.3) | 7 (25.0) |
| Nasal edema | 6 (75.0) | 6 (75.0) | 1 (12.5) | 3 (37.5) | 0 (0.0) | 1 (8.3) | 0 (0.0) | 6 (11.3) | 1 (3.6) |
| Pharyngeal erythema | 2 (25.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 3 (5.7) | 0 (0.0) |
| Epistaxis | 1 (12.5) | 0 (0.0) | 0 (0.0) | 1 (12.5) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) |

[a] Data pooled from a phase 1 safety and immunogenicity study (N = 12) [16], a placebo controlled vaccination study (M. Riddle, personal communication, N = 10) and a placebo controlled vaccination-challenge study (C. Harro, personal communication, N = 31).

[b] Data pooled from a placebo controlled vaccination study (M. Riddle, personal communication, N=7) and a placebo controlled vaccination-challenge study (C. Harro, personal communication, N = 21)

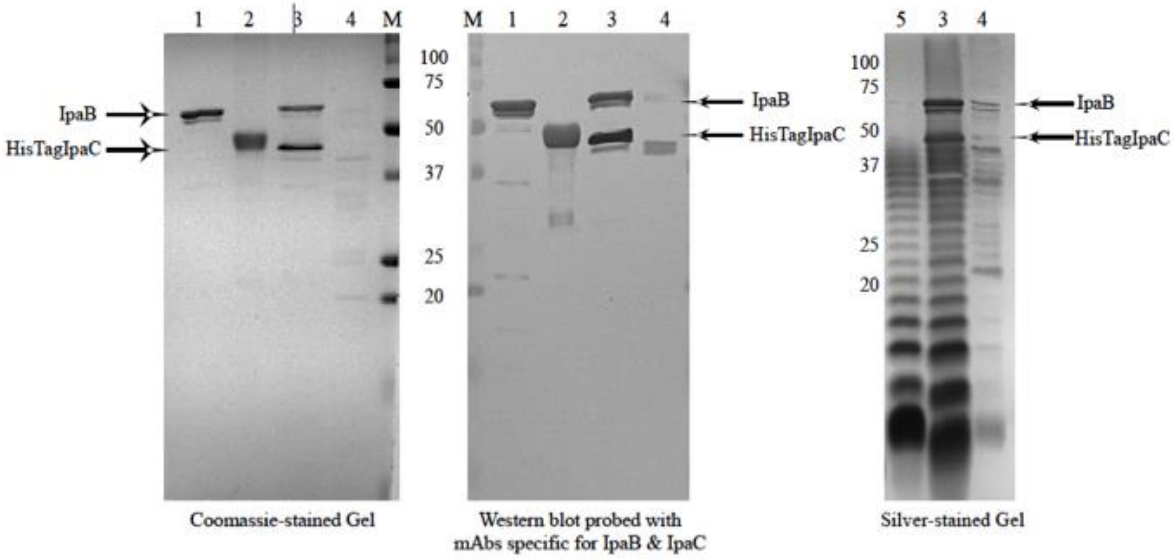
## 5.2. Artificial Invaplex

Despite the excellent safety profile of *S. flexneri* 2a Invaplex$_{NAT}$, inconsistent immune responses and lack of protection from disease in a human challenge model highlighted the need for further vaccine development. The composition of Invaplex$_{NAT}$ includes a high molecular weight complex and several undefined proteins. The high molecular weight complex contains the known major Shigella-virulence factors and immunogens, including IpaB, IpaC, and LPS and is associated with protective activity of *S. flexneri* 2a Invaplex$_{NAT}$. Other, undefined proteins are not considered active ingredients of the vaccine and, therefore, may have diluted the potency of key antigens (IpaB, IpaC and LPS) [11] in the high molecular weight complex and thus undermining the optimal potential immune response solicited. In addition, the undefined components may contribute destabilizing activities, such as protease activity, that may have contributed to inconsistent immunogenicity of the *S. flexneri* 2a Invaplex$_{NAT}$ vaccine observed over time. Subsequently, to improve the overall definition and immunogenicity of the high molecular weight component of Invaplex the development of a more refined vaccine [*S. flexneri* 2a artificial Invaplex (Invaplex$_{AR}$)] commenced.

### 5.2.1. Synopsis: *S. flexneri* 2a Invaplex$_{AR}$

Briefly, recombinant IpaB and IpaC proteins were purified from separate recombinant *Escherichia coli* [17, 18]. LPS was purified from *S. flexneri* 2a strain 2457T by the Westphal procedure [19]. The individual components (IpaB, IpaC and LPS) were assembled into the high molecular weight *S. flexneri* 2a Invaplex$_{AR}$ complex, which was subsequently purified by ion-exchange size exclusion chromatography using conditions similar to that used to purify *S. flexneri* 2a Invaplex$_{NAT}$. The vaccine was then sterile filtered, stored frozen, and available for vaccine studies. Initial studies compared *S. flexneri* 2a Invaplex$_{NAT}$ to *S. flexneri* 2a Invaplex$_{AR}$. Figure 1 shows the overall composition of each vaccine and demonstrates that the research grade *S. flexneri* 2a Invaplex$_{AR}$ has only 3 components (IpaB, IpaC, and LPS) at much higher proportions on a weight basis as compared to *S. flexneri* 2a Invaplex$_{NAT}$.

**Figure 1: Comparison of *S. flexneri* 2a Invaplex$_{AR}$ and *S. flexneri* 2a Invaplex$_{NAT}$ by SDS-PAGE**

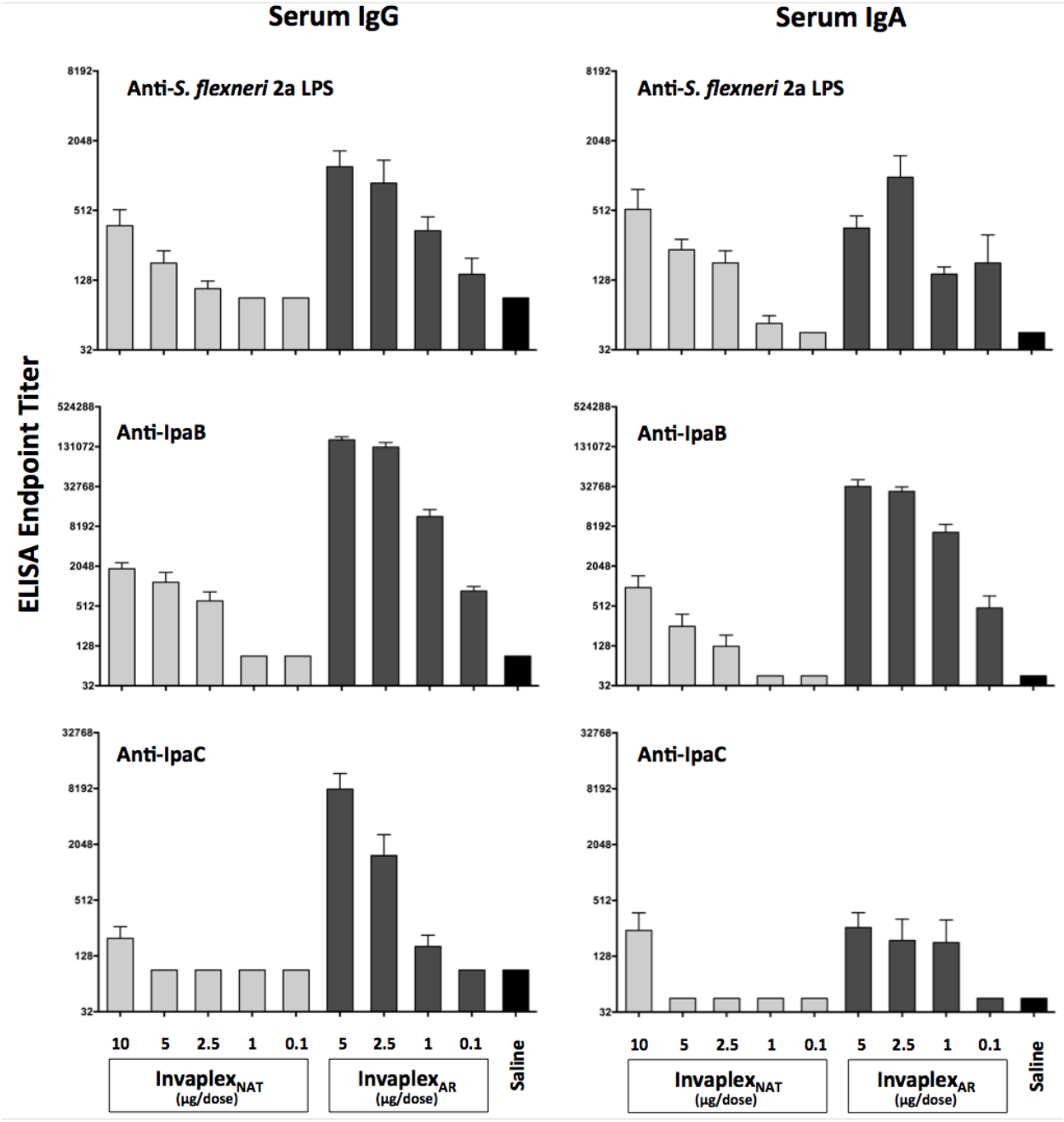



*S. flexneri* 2a Invaplex$_{AR}$ is assembled from purified IpaB (lane 1, 1 µg), purified IpaC (lane 2, 1 µg), and purified *S. flexneri* 2a LPS (lane 5, 2.5 µg). After assembly the complex is isolated by ion-exchange chromatography. The purified Invaplex$_{AR}$ (5 µg) is in lane 3. For comparison purposes Invaplex$_{NAT}$ (5 µg) is also run on these gels (lane 4). The panel on the left is a Coomassie stained SDS-PAGE gel, the middle panel is a western blot probed with monoclonal antibodies specific for IpaB and IpaC and the panel on the right is a silver-stained gel that is used to show both the protein and LPS components of the Invaplex product. In this gel, HTIpaC (HisTag-IpaC) is used; in later stages of Invaplex$_{AR}$ development, only non-his-tagged IpaC was used. The Invaplex$_{NAT}$ preparation consists of many proteins in addition to IpaC and IpaB thereby reducing the proportion of the key antigens (IpaB, IpaC, and LPS) in the overall product composition.

### 5.2.2. Comparative Efficacy of *S. flexneri* 2a Invaplex$_{AR}$ (research grade) and *S. flexneri* 2a Invaplex$_{NAT}$

To demonstrate that the increased proportions of the key antigens in *S. flexneri* 2a Invaplex$_{AR}$ resulted in improved immunogenicity groups of mice (5 per group) were intranasally immunized on Day 0, 14, and 28 with a research grade lot of *S. flexneri* 2a Invaplex$_{NAT}$ (0.1, 1, 2.5, 5, or 10 µg), and a research grade lot of *S. flexneri* 2a Invaplex$_{AR}$ (0.1, 1, 2.5, or 5 µg) or saline. Blood was collected on Day 42 and analyzed by enzyme-linked immunosorbent assay for anti-LPS, IpaB and IpaC IgG and IgA endpoint titers. The mean endpoint titers + 1 standard error are shown in Figure 2. The results clearly demonstrate that lower dose amounts of Invaplex$_{AR}$ could be used to stimulate higher titers than that possible with *S. flexneri* 2a Invaplex$_{NAT}$.

**Figure 2: Comparative Immune Responses in Mice Receiving *S. flexneri* 2a Invaplex$_{AR}$ or *S. flexneri* 2a Invaplex$_{NAT}$**




## 5.3. Development of *S. flexneri* 2a Invaplex$_{AR\text{-}DETOX}$

While Invaplex$_{AR}$ has demonstrated immunogenicity when administered by IN route, there is concern that the LPS moiety of the vaccine could lead to excessive reactogenicity when used parenterally. Additionally, intranasal immunization may not be practical or acceptable as a route of administration to infants and children. In light of this concern, the "detoxified" Invaplex$_{AR\text{-}}$

$_{DETOX}$ was developed using genetically-attenuated *Shigella flexneri* lacking late acyl transferases (ΔmsbB), resulting in lipid A mutants that are underacylated. This underacylated LPS is used in combination with recombinant IpaB and IpaC in similar fashion as Invaplex$_{AR}$ under cGMP. Underacylated LPS induces less pro-inflammatory cytokine release from macrophages in vitro, and Invaplex$_{AR\text{-}DETOX}$ has been demonstrated to induce lower reactogenicity as compared to Invaplex$_{AR}$ after ID vaccination in mice, while still inducing the same levels of *Shigella*-specific antibodies in mice and guinea pigs.

## 5.4. Nonclinical Toxicology

A nonclinical toxicology study was conducted in New Zealand White (NZW) rabbits to evaluate the toxicity and local site reactogenicity of Invaplex$_{AR\text{-}DETOX}$ when administered intramuscularly at up to 25 µg protein content. This study also included an adjuvant, dmLT, which is not included in the clinical protocol due to formulation challenges. This study was conducted in compliance with Good Laboratory Practices.

As shown in Table 6, a total of 88 (44 male and 44 female) NZW rabbits were randomized into each designated group. Animals were administered four biweekly intramuscular doses of saline (Group 1), 10 µg Invaplex$_{AR\text{-}DETOX}$ + dmLT (Group 2), 25 µg Invaplex$_{AR\text{-}DETOX}$ (Group 3), or 25 µg Invaplex$_{AR\text{-}DETOX}$ + dmLT (Group 4) on days 1, 15, 29, and 43. Animals were followed for standard vaccine toxicology endpoints, including detailed physical exams, ophthalmological exams, body weight measurements, evaluation of body temperature and assessments of dose site inflammation following each dose, and clinical pathology. Six animals of each sex from each group were euthanized and underwent full necropsy on study day 45 (2 days post final vaccination). The remaining five animals per sex from each group were euthanized and underwent a full necropsy on day 73 following a 28 day recovery period. Microscopic evaluation of major organ systems and local injection sites was conducted on all animals sacrificed on study day 45. Only injection sites were evaluated microscopically from those animals sacrificed on study day 73.

**Table 6: Nonclinical Toxicology Study Design**

| Group | Invaplex$_{AR\text{-}DETOX}$ Dose | dmLT Dose | Number of Animals | |
|---|---|---|---|---|
| | | | Termination Day 45 | Termination Day 73 |
| 1 | N/A | N/A | 6M/6F | 5M/5F |
| 2 | 10 µg | 0.1 µg | 6M/6F | 5M/5F |
| 3 | 25 µg | N/A | 6M/6F | 5M/5F |
| 4 | 25 µg | 0.1 µg | 6M/6F | 5M/5F |

Invaplex$_{AR\text{-}DETOX}$ was well-tolerated following four bi-weekly doses, with no adverse effects observed in NZW rabbits at either 10 or 25 µg. Non-adverse apparent effects of treatment included decreased food consumption without correlating changes in body weights, gross and microscopic inflammation at the injection sites, temporary shifts in clinical pathology indicating an immune and/or inflammatory response following vaccination, increased relative and absolute spleen weights, and microscopic changes in the spleen and sciatic nerve. These findings were consistent with the pharmacology of the vaccine and/or were not associated with a chronic or

disease effect. While Invaplex$_{AR\text{-}DETOX}$ appears to induce an acute systemic inflammatory response, this response appears to be in a dose-related manner.

## 6. Trial Objectives

**Primary:** Evaluate the safety of a detoxified *Shigella flexneri* 2a Invaplex$_{AR}$ (Invaplex$_{AR\text{-}DETOX}$) administered by intramuscular immunization.

**Secondary:** Evaluate immune responses (serum IgG/IgA and ALS IgG/IgA) following IM immunization with Invaplex$_{AR\text{-}DETOX}$.

**Exploratory:**

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

## 7. Hypotheses

**Safety:** One or more of the proposed dosing regimens will have an acceptable frequency and severity of adverse events (AEs).

**Immunogenicity:**

- IM administration of Invaplex$_{AR\text{-}DETOX}$ will induce serum IgG antibody responses to *S. flexneri* 2a Invaplex in ≥50% of subjects in at least one of the study groups.
- IM administration of Invaplex$_{AR\text{-}DETOX}$ will induce antibody responses to either IpaB or IpaC in ≥50% of subjects in at least one of the study groups.

## 8. Clinical Study Design

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 7. 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 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.

**Table 7: Clinical Trial Design for the Proposed Phase 1 Study with *S. flexneri* 2a Invaplex$_{AR\text{-}DETOX}$**

| Cohort | Group | n | Route | Invaplex$_{AR\text{-}DETOX}$ (µg) |
|---|---|---|---|---|
| A | A-1 | 16 | IM | 2.5 |
| | A-2 | 4 | IM | Placebo |
| B | B-1 | 16 | IM | 10 |
| | B-2 | 4 | IM | Placebo |
| C | C-1 | 16 | IM | 25 |
| | C-2 | 4 | IM | Placebo |

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 (see Table 8). 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 study time and events schedule is shown in Table 8.

## Table 8: Clinical Protocol Time and Events Schedule

| Study Event | Screening | -7[1] | 1 | 2 | 8 | 22 | 23 | 29 | 43 | 44 | 50 | 57 | 71[2] | 223 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 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 Examination | 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 | |
| Vaccination | | | 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 vaccination days include pre- and post-vaccination complete assessments including targeted physical exams (volunteers will be observed for 30 minutes post-vaccination), baseline exam, and physical assessment.
[5] Volunteer diaries post-vaccination will begin day of vaccination through the 7-day post-vaccination 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 collection may include saliva.
[9]Day 223 assessments will be performed via telephone.

## 8.1. Screening and Study Schedule

The screening visit is scheduled -60 to -9 days before the first vaccination. Fully informed, written consent must be obtained from each subject prior to conducting any study procedure. Two consent forms will be signed; a consent form for study participation and a separate consent form for HIV testing. To assess understanding, a comprehension assessment will be administered after the subjects sign the study participation written consent. The following evaluations/procedures will be carried out:

- Oral and/or video presentation of clinical trial design, risks and study schedule
- Review of informed consent document
- One-on-one discussions with principal investigator or subinvestigator
- Signature of informed consent document (see section 9.2 for informed consent process)
- Comprehension test (minimum of 70% accuracy required for participation, two attempts allowed)
- Medical history – between Days -60 to -9
- Physical examination – between Days -60 to -9
- Vital Signs – between Days -60 to -9
- Screening laboratory analysis (allowable time period for accepting test):
  - Complete blood count (between Days -60 to -9)
  - Serum transaminases (ALT/AST) (between Days -60 to -9)
  - Na, K+, CL-, $HCO_3$, glucose, BUN and creatinine (between Days -60 to -9)
  - Total serum IgA (between Days -60 to -9)
  - Pregnancy test (urine -hCG) for women, as well as 0-24 hours before each vaccination
  - HLA-B27 (between Days -60 to -9)
  - Serology screening (Anti LPS Antibody) for prior *Shigella* exposure (between days -60 to -9)
  - Serum HIV 1/2 antibody (between days -14 to -2)
  - Hepatitis B surface antigen (HBsAg) (between days -14 to -2)
  - Hepatitis C virus Ab (between days -14 to -2)

Additionally, approximately 7 days prior to vaccination (allowable range: Days -14 to -2), subjects will have a follow-up medical history and brief physical exam to ensure ongoing eligibility. If the initial screening is within the -14-day window, it can count as both the initial screening and pre-vaccination visit (and all screening activities will be conducted at the one visit). Blood, saliva, and stool will be collected for baseline serology testing. Additional

instruction will be reviewed with each subject to include review of the study visit schedule, preparation procedures for saliva collection, and procedures for stool collection and storage. The following screening laboratory analyses will be carried out (below tests will not be repeated if the initial screening visit occurs during the day -14 to -9 window):

- Repeat complete blood count
- Repeat serum transaminases (ALT/AST)
- Repeat Na, K+, CL-, $HCO_3$, glucose, BUN and creatinine
- Serum HIV 1/2 antibody (between Days -14 to -2)
- Hepatitis B surface antigen (HBsAG) (between Days -14 to -2)
- Hepatitis C virus (anti-HCV antibody) (between Days -14 to -2)

A screening test for any of the above assessments may be repeated in the event of a laboratory error (i.e., hemolyzed sample) or in the event the screening physician believes: 1) the laboratory test has identified a normal variant of a healthy state (i.e., below normal hemoglobin in a menstruating female, or cyclic reductions in absolute neutrophil count in subjects of African descent) and 2) the variant is no more than a Grade 1 abnormality. This repeat screening applies to the initial screening tests [(executed between -60 to -9 days) and the repeat screening labs if executed (between days -14 to -2)]. If a repeat screening blood test is performed, only the results of the second test will be used to determine eligibility.

The WRAIR Clinical Trials Center (CTC) may use a screening protocol in recruiting volunteers for this study. All screening procedures outlined in this section may be completed under this screening protocol.

During this screening process, the laboratory assays will be reviewed to determine if any clinical laboratory abnormalities exist that would preclude study participation. Subjects who have only 1 mild (grade 1) abnormality may be included if the PI determines that their participation will not present undue risk to the subject. Subjects with 2 or more mild abnormalities may be included in the study only with the consensus of the PI and the research monitor. Subjects with clinical laboratory abnormalities of greater than mild severity will not participate in this clinical trial. The clinical toxicity grading scale that will be used as a guideline is based on the scale used by the Division of AIDS (DAIDS) for adverse events and the guidance from the US Food and Drug Administration (FDA) Center for Biologics Evaluation and Research. If any additional safety labs are performed, either scale may be utilized.

## 8.2. Randomization

Within each cohort, 20 subjects will be randomly assigned to one of 2 different treatment groups using block sizes of 5. 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.

Subsequent dose-escalating cohorts will be randomized after review of safety data including 7 days after the final vaccination of the previous cohort. Depending on the safety profile, the PSRT will make a recommendation to the sponsor and investigator to proceed or not with the vaccination of the next cohort at a higher dose level.

Participant randomization will be generated from the Advantage eClinical system based on the enrollment sequence in the treatment table, therefore the randomization plan will also provide the maximum number of sequence numbers by site. An Emmes statistician will write code using SAS and the seed(s) for the random number generator will be documented. When a participant is randomized in Advantage eClinical only the cohort, coded treatment assignment and the sequence number will display. A second Emmes statistician will conduct and document quality control (QC) of the randomization plan, treatment table and all other related documents using the statistical considerations in the protocol. Once the treatment table has been finalized, the Emmes Systems Programmer will transfer the treatment table into the Advantage eClinical environment and the Emmes Project Manager will approve the treatment table. The person performing vaccine formulation will be provided a list of the coded treatment numbers with their corresponding unblinded treatment assignments, as well as the sealed back-up manual randomization list.

## 8.3. Blinding

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.

## 8.4. Unblinding

In the event of emergency, the site Investigator may require that the blind be broken for the subject experiencing the emergency when knowledge of the subject's treatment assignment may influence the subject's clinical care. Emergency unblinding can occur by contacting Emmes during business hours or by contacting the unblinded vaccine formulation expert after business hours. Every effort will 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 vaccinations. The decision to unblind will be communicated to any 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.

## 8.5. Vaccination

After initial screening and informed consent, eligible volunteers will be vaccinated with *S. flexneri* 2a Invaplex$_{AR\text{-}DETOX}$ Lot 1972 or placebo (saline). The vaccine will be administered IM on Days 1, 22, and 43. The investigational product administration procedure is outlined in detail in the Manual of Procedures (MOP). Briefly, the IM vaccination site (deltoid muscle) will be pre-treated with an alcohol pad and the vaccine will be administered using a 1-inch needle. Approximately 100 µL of appropriately formulated vaccine product or placebo will be administered. Subjects will be observed for at least 30 minutes post-vaccination and given symptom diaries to record local and systemic reactions for 7 days post vaccination for each dose. After the 30-minute observation period, an assessment will be performed including vital signs and the recording of all adverse events. Subjects will return approximately 24 hours after vaccination for a follow-up examination. For each subsequent immunization, the injection will be given in alternate deltoid regions from the previous vaccination. An exception may be needed if there is pain/tenderness, erythema, edema, pruritus, induration, or rash at the planned site of vaccination. In this case, the Principal Investigator, following discussion with the Independent Research Monitor, may exercise the discretion to administer the vaccination on the same arm as the previous vaccination. The site of vaccine administration will be recorded in the source documents.

### 8.5.1. Vaccination Follow-Up

The timing and number of follow-up visits are delineated in the Time and Events Schedule (Table 8) and are scheduled to coincide with vaccination or specimen collection visits. These visits may include a brief history, targeted exam, blood, stool, and salivary specimen collection, and a reminder to the volunteer to contact the investigator if s/he experiences any medical problems. Subjects will return approximately 24 hours and 7 days after vaccination for follow-up.

Approximately 6 months from final vaccination all subjects will be contacted via telephone to assess for a final safety assessment. A history for any additional serious new diagnoses or hospitalizations will be solicited. Three attempts will be made and documented; after 3 unsuccessful attempts a certified letter and/or email will be used to request the subject to contact the study team.

## 8.6. Endpoints

### 8.6.1. Safety

Safety monitoring will be undertaken using in-person symptom surveillance, symptom diaries, and targeted physical exams. Solicited adverse event monitoring will survey and specifically inquire about symptoms listed in section 11.6.2. Abnormalities will be described in detail in order to monitor progress. This will include the name of the adverse event, the date and time of onset and offset (duration), severity, and Principal Investigator determination of relationship to the investigational vaccine. Safety monitoring labs will also be performed on Days 8, 29, and 50. Clinical outcomes will be prospectively defined and actively surveyed during the study. The study procedures are detailed in Table 8.

Clinical definitions will be used to grade the severity of symptoms in accordance to the severity scale included in section 11.8.3.

### 8.6.2. Immunology

A secondary objective is to assess IgG and IgA serologic and Antibody in Lymphocyte Supernatant (ALS) responses to *S. flexneri* 2a Invaplex using methods previously established. Seroconversion will be defined as a ≥ 4-fold increase in endpoint titer between pre- and post-vaccination samples. Peripheral Blood Mononuclear Cells (PBMCs) will be collected to determine antigen-specific IgG and IgA titers in ALS samples. An ALS response is defined as ≥ 4-fold increase in antibody titer at any point post-vaccination as compared to baseline titers. For serologic and ALS assays, high-titer specimens will be included on each plate to track day-to-day interassay variation. For each antigen, pre- and post-vaccination serum samples will be assayed side-by-side. The antibody titer assigned to each sample will represent the geometric mean of duplicate tests. Reciprocal endpoint titers less than the starting dilution of the assay will be assigned a value of half the starting dilution for computational purposes.

Secondary immunologic evaluations (an exploratory objective) may include serologic and ALS responses to additional antigens (*S. flexneri* 2a LPS, IpaB, IpaC), additional IgG subclasses, IgM responses, and serum bactericidal antibody (SBA) titers against *S. flexneri* 2a 2457T (responders defined as ≥ 9-fold increase in SBA titer at any point post-vaccination compared to baseline titers). Stool samples will be collected to assess antigen-specific fecal Ig responses. A fecal response will be defined as a ≥ 4-fold increase from baseline.

Samples will also be collected, processed, and archived for potential future analysis as follows. PBMCs will be collected for antigen-specific memory B/T cell analyses, antibody secreting cell (ASC) assay, and α4β7+ B cells. Concurrently, saliva samples may be collected to assess mucosal IgA and IgG antibody responses. CRP responses in serum after initial vaccination will be collected for exploratory correlation with immune responses and AEs.

## 8.7. Investigational product

### 8.7.1. Invaplex$_{AR\text{-}DETOX}$

*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 2mL glass vials, stoppered and sealed, and stored at -80 ± 10°C.

**Figure 3: Label from a vial of Invaplex$_{AR\text{-}DETOX}$, Lot 1972**

***Shigella flexneri* 2a WR30 Artificial**
**Invaplex (Invaplex$_{AR\text{-}Detox}$)**
**BPR No.: BPR-1214-00** **Lot No.: 1972**
Contents: 0.6 ml ± 0.06 Storage: -80 ± 10°C
Caution: New Drug - Limited by Federal (or United States) law to investigational use.
Date of Mfg.: 15 Jan 16
Manufactured By: WRAIR, Silver Spring, MD 20910

### 8.7.2. Investigational Product Preparation

On the day of vaccination, the product will be used to formulate the appropriate vaccine preparations for the clinical groups. The vaccine will be used within 6 hours of preparation. Prepared vaccine or open vials will not be saved for use on another study day. Any remaining vaccine product will be properly disposed of in accordance with the MOP. Vaccine formulation for each of the groups is described in detail in the MOP.

### 8.7.3. Investigational Product Accountability

The sponsor is responsible for authorizing the release for distribution of the investigational products to the study site. The NMRC Enteric Diseases Department and/or WRAIR will arrange for shipment, under appropriate conditions, of the investigational products to the clinical site. At the WRAIR CTC, investigational product inventory logs will be maintained in the accountability files. The Release for Use Memo signed by the sponsor's representative gives permission to the WRAIR Pilot Bioproduction Facility to release product to the PI. After the investigational product is distributed, the PI is responsible for and will maintain logs of investigational product receipt, storage, reconstitution, accountability by subject, and investigational product remaining before final disposition. These logs will be maintained in the regulatory files at the WRAIR CTC. The PI may delegate, in writing, this responsibility to another individual, but the PI is ultimately responsible for the investigational product and its proper storage upon receipt at the study site until it is transferred back to the sponsor or designee, utilized as directed by the sponsor or destroyed. Any excess formulated vaccine remaining from vaccination day will be disposed of or used for research purposes as authorized by the sponsor. All used or partially used investigational product and empty vials will be destroyed or used for nonclinical research purposes as per the MOP.

## 8.8. Duration of Subject Participation

Screening of volunteers will occur during a period of up to 60 days prior to initial vaccinations. Subjects will receive 3 vaccinations on Days 1, 22, and 43. Clinical follow-up will occur as delineated in the Time and Events schedule (Table 8). A phone survey will occur at approximately 6 months after the final vaccine dose. This phone survey will specifically inquire if the subject has experienced any new serious health problems or hospitalizations since their last study follow-up. Inclusion criteria require that a subject be available for the required follow-up period and scheduled clinic visits. Therefore, no military subjects will be enrolled in the study if a known deployment is to conflict with his or her involvement. If there is an unexpected deployment, efforts (e.g., telephone, e-mail, command mail) will be undertaken to obtain safety follow-up data per the protocol schedule.

## 8.9. Dose Escalation Criteria

The decision to advance to the next dose level will be based solely on the safety assessment. A dose level with no occurrence of stopping criteria will prompt moving to the next higher level. The Protocol Safety Review Team (PSRT) comprised of the Principal Investigator, the PATH Medical Officer, and the Independent Research Monitor will review blinded safety data for the recommendation to proceed with dose escalation.

## 8.10. Stopping Criteria

The study will use a dose-escalation design to evaluate increasing doses of Invaplex$_{AR\text{-}DETOX}$.

The PI, along with the PSRT, may determine a subject's local site reactions warrant discontinuation of subsequent vaccinations for that subject. If it is determined that a subject will not continue with future vaccine doses, they may remain enrolled in the study for follow-up.

If any of the following events occurs, administration of investigational product will be discontinued for the group until a thorough review of the event is undertaken by the investigators and the PSRT:

- The occurrence of one serious adverse event determined to be related to the investigational vaccine (definitely, probably, or possibly) at any time post-vaccination.
- Severe diarrhea, defined as six or more liquid stools within 24 hours of vaccination.
- A severe local rash, defined as one that makes the subject unable to perform normal daily activities and which is not attributable to another cause.
- Systemic rash, including but not limited to generalized urticarial, generalized petechiae, or erythema multiforme, occurring in two or more subjects in a group will result in stopping further vaccination in subject's group.
- One vaccine-related serious or unexpected AE evaluated by the principal investigator (PI), Independent Research Monitor, and sponsor and determined to be an unacceptable risk to the health and safety of other investigational product recipients.
- If a subject experiences grade 2 or greater hypotension in the 30 minutes following vaccination, they will not be eligible to receive subsequent vaccinations.

Further vaccination, in accordance with the protocol, may be resumed with the concurrence of the PRST, sponsor's representative, the PI, and the US Food and Drug Administration (FDA).

An interim analysis for each cohort will be prepared and reviewed to include all safety data through seven days after the third vaccination dose for each cohort. This 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. In addition to the interim safety analyses, the PI, sponsor's representative along with the PSRT will stop the study until further review by the PSRT if any of the following criteria that are thought to be definitely, probably, or possibly related to the investigational vaccine are identified:

- One or more SAEs, including grade 4 adverse events or laboratory abnormalities.
- Two or more grade 3 adverse events or laboratory abnormalities of the same type.
- One or more grade 3 laboratory abnormalities.
- Two or more grade 2 laboratory abnormalities.

## 8.11. Identification of Data to Be Recorded on Case Report Forms

The electronic case report form (eCRF) data will be entered from source documentation. No source data will be recorded directly in eCRF (i.e., without prior record of data). These data will be consistent with the source documents or the discrepancies will be explained. No personally

identifying data will be recorded on eCRFs. The investigator is ultimately responsible for the accuracy of data transcribed on the eCRF. Data monitoring and management will be performed in the electronic data capture (EDC) database system by the study monitor and the designated data management group. A detailed data management plan will be written and approved by the study team and the PI prior to study start. All updates to the data management plan must be approved before study closeout and database lock.

## 8.12. Known and Potential Risks and Benefits to Human Subjects

### 8.12.1. Risks/Discomfort to Subjects and Precautions to Minimize Risk

Outlined as follows are anticipated and unexpected adverse reactions with a brief description of procedures to ameliorate risks and symptoms. In general, dose escalation is being performed to ensure an acceptable safety record for IM administration of the vaccine product in this study. Prior to proceeding with the next dose in the next group of subjects, all safety data will be reviewed by the PSRT, and a determination will be made as to whether the study can continue to enroll subsequent cohorts (at the dose escalation planned) based on the observed safety record from the preceding dose level. In addition to this overall safeguard, individual risks will be closely monitored and any AEs will be quickly identified and managed.

#### 8.12.1.1. Local Reactions

Local reactions at the vaccination site are expected from IM immunization. The IM route of vaccination, in general, has been shown to be a safe and effective route of administration. There may be some mild discomfort and irritation from an IM vaccination from the initial injection. These are typically mild and transient in nature. In addition, there is the possibility of local erythema, pruritus, swelling, and/or induration that appears over time. In addition, subjects may develop temporary swelling in the lymph nodes under the arm where vaccination occurred.

#### 8.12.1.2. Systemic Reactions

Any vaccine may be associated with a wide range of systemic reactions, such as fevers, constitutional symptoms (fatigue, malaise, appetite change), and gastrointestinal symptoms (diarrhea, abdominal pain) as well as others to include allergic reactions (bronchospasm, urticaria, anaphylaxis). The frequency and type of systemic symptoms will be assessed and analyzed with respect to study product.

#### 8.12.1.3. Pregnancy

During initial screening and prior to each vaccination, a urine pregnancy test will be performed. Subjects with a positive test will be excluded from the study. A final urine pregnancy test will be conducted during the last clinic visit. Female subjects of childbearing potential must agree to use an effective method of birth control (birth control pills, injection hormonal contraceptive, implant hormonal contraceptive, hormonal patch, IUD, sterilization by hysterectomy or tubal ligation, spermicidal products and barrier methods such as cervical sponge, diaphragm, or condom) during the study and 3 months following the last vaccination. Abstinence is acceptable. While female subjects will not be required to disclose their method of birth control, they will be counseled during the initial screening on acceptable forms of birth control required for study enrollment. At each visit, female subjects will be reminded that they must agree to use effective

contraception to remain in the study. Female subjects should not become pregnant during the study and for at least 3 months after the last vaccine dose.

#### 8.12.1.4. Lactation

Risks to nursing infants are unknown at this time; breastfeeding females will be excluded from this study.

#### 8.12.1.5. Venipuncture

Blood sampling carries a minimal risk of minor discomfort and the possibility of minor bruising at the site of the needle puncture and, rarely, the possibility of infection at the needle puncture site.

#### 8.12.1.6. Allergic Reaction

As with any Investigational New Drug (IND) product administration and no matter what precautions are taken, there is always the risk of a serious, or even life-threatening, allergic reaction. Although anaphylactic reactions are not anticipated, as with any vaccination, the rare possibility exists. For this reason, all subjects will be observed at the study site (WRAIR CTC) for a minimum of 30 minutes following vaccine dosing. Appropriate emergency equipment (e.g., automated external defibrillator, air-shields manual breathing unit bag) and medication [e.g., antihistamines and adrenaline (epinephrine)] for initial treatment of an allergic reaction will be available at the WRAIR CTC whenever immunizations are given. This equipment is available to handle emergencies such as anaphylaxis, angioedema, bronchospasm, and laryngospasm. Existing staff standard operating procedures at the WRAIR CTC are also in place for these events.

#### 8.12.1.7. Theoretical Risks

Post-infection reactive arthritis, characterized as an asymmetric, oligoarticular arthritis commonly involves the knees, ankles, or wrists is known to occur following *Shigella* infection. Some data suggests that the risk of reactive arthritis following shigellosis may be higher in persons carrying the Human Leukocyte Antigen (HLA)-B27 antigen [20]. There is enough uncertainty as to the etiology of arthritis, that a specific entry criteria related to this theoretical risk is included and will exclude volunteers with a personal or immediate family history of inflammatory arthritis or HLA-B27 positivity.

#### 8.12.1.8. Unknown Risks

As with all research, there is the remote possibility of risks that are unknown or that cannot be foreseen based on current information. This would include late effects that have been seen with some vaccines.

### 8.12.2. Alternatives to This IND Product or Study

At this time, there are no known alternative, licensed vaccines directed at *Shigella*. An alternative choice is not to participate in this study.

### 8.12.3. Intended Benefit for Subjects

There is no benefit that can be guaranteed to volunteers for participating in this research study. However, there is potential societal benefit of the development of an effective *Shigella* vaccine.

### 8.12.4. Risks to Study Personnel and the Environment

The principal risk in the clinical setting is in the handling of needles that may be contaminated and the attendant risks including hepatitis, human immunodeficiency virus (HIV), and other human pathogens. Adherence to standard operating procedures (SOPs) for working with infectious agents and universal precautions will reduce the risk of exposure.

There are no known risks to the environment other than those associated with the generation of biohazardous waste attendant to vaccination of humans. All biohazardous waste will be disposed of as stipulated by local, state, and federal regulations and in accordance with study-site SOPs.

## 8.13. Route of Administration, Dosage Regimen, Treatment Period, and Justification

On days 1, 22, and 43 subjects will be administered Invaplex$_{AR\text{-}DETOX}$ or placebo. Each subject will receive the same formulation at each vaccination dependent upon group assignment. Dose formulations will be prepared each day of immunization at the clinical site using the Invaplex$_{AR\text{-}DETOX}$ drug product. Because Invaplex$_{AR\text{-}DETOX}$ has not been previously administered to humans, limited information is available to estimate the dose needed to induce suitably robust immune responses while maintaining an acceptable safety profile. Guinea pigs have been immunized intranasally with 25 µg or intramuscularly with 1, 5, and 25 µg of cGMP Invaplex$_{AR\text{-}DETOX}$. The vaccine was well-tolerated and induced a robust immune response. This study is designed as a placebo-controlled, dose-escalating study starting with the lowest dose of Invaplex$_{AR\text{-}DETOX}$ (2.5 µg) and progressing upwards through higher doses up to 25 µg. Following completion of each of the vaccination series, an interim safety report will be compiled and a dose-escalation decision will be made in concert with the PSRT. If the vaccine is safe and immunogenic, future studies will evaluate the preliminary efficacy of this vaccine using a homologous enteric *S. flexneri* 2a strain in a human challenge model. The overall goal of the current *Shigella* development plan is to create a broadly protective multivalent *Shigella* vaccine from a combination of Invaplex vaccines encompassing the conserved invasin proteins and the combination of serotype specific LPS for each proposed strain to integrate. The proposed multivalent vaccine would integrate the major *Shigella* serotypes responsible for human disease: *S. flexneri* 2a, *S. flexneri* 3a, *S. flexneri* 6, and *S. sonnei*.

## 8.14. Compliance Statement

The study will be conducted according to the protocol and in compliance with International Council on Harmonisation (ICH) Good Clinical Practice (GCP), Belmont Principles, and other applicable regulatory and DoD requirements. All identified study personnel will be trained to perform their roles and will carry out their responsibilities in accordance with ICH GCP guidelines and clinic site study procedures.

## 8.15. Study Population

In this initial study, it is important to restrict enrollment to a small number of healthy subjects without factors that may limit or confound assessment of AEs and vaccine-induced immune

response. The study population will consist of healthy male and nonpregnant, nonlactating female subjects, ages 18-50 years (inclusive), from the greater Washington, DC and Baltimore, Maryland areas, who can give informed consent and understand the risks and benefits of the study and are willing to comply with all protocol procedures and time commitments. A maximum of 60 subjects will be enrolled in this study. Four alternates per cohort may be asked to go to the clinic on vaccination days in the event a planned subject cannot/does not enroll in the study.

### 8.16. Study Site

The study will be conducted at the WRAIR CTC, Silver Spring, Maryland. Immunologic assays will be conducted at the subunit enteric vaccines and immunology laboratory of the WRAIR, Silver Spring, Maryland.

## 9. Selection and Withdrawal of Subjects

### 9.1. Recruitment of Subjects/Study Population

Healthy adults, both males and nonpregnant 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 of recruitment, printed media, handouts, and briefs will have IRB approval prior to being used.

When a subject calls the WRAIR CTC recruitment office and discloses an interest in the study, the recruitment staff will discuss details of the trial from a prepared, IRB-approved script. If the subject is still interested, contact information will be obtained and an appointment for briefing/screening will be arranged.

Up to 60 subjects will be recruited for enrollment in this Phase 1 trial. Additionally, there will be up to 4 alternates recruited for each cohort.

### 9.2. Informed Consent Process

All subjects will undergo an informed consent process consisting of a detailed informational presentation of study-related material using a pre-recorded brief. Following the briefing, one of the study investigators will reiterate the purpose, procedures, and risks of the study as needed and answer any questions. This process will take place at the WRAIR CTC immediately preceding the screening procedures.

During the briefing session, an investigator or designee will brief prospective subjects using the briefing slides. The briefing may be provided by a pre-recorded slide set. An investigator will be available to answer any questions raised during the session. Following the briefing, the coordinator or designee will provide the subject with ample time to read the consent and will

answer any questions. The subject will be asked to sign the consent, as will the investigator conducting the informed consent discussion. The subject will be allowed to take the consent document home to consider and discuss it with others and can return it to the WRAIR CTC at a later time to sign it. After signing the consent (to include future use of specimens), the subject will take a comprehension test ("open book" format). Any questions missed will be explained to the subject, and the subject's questions will be answered. The test is administered to aid study personnel in identifying gaps in understanding. If the subject appears not to understand the content of any of the questions, one of the study facilitators will be informed and must document whether the subject understands the risks, benefits, procedures, and time commitment as well as the answers to the questions that he or she missed. Any subject who, in the opinion of the PI, does not understand the study well enough to consider his or her consent truly informed will be excluded. The subject will be given one additional opportunity to take the comprehension test. A passing score of at least 70% will be required to participate in the study.

The informed consent process will include a recruitment script, briefing material, and an informed consent document. Throughout the study, all questions and concerns of the subject will be addressed. If the subject has additional concerns, the research monitor will be available to answer any medical questions, and the NMRC Office of Research Administration is available to answer any human use-related concerns.

No study procedures will occur before the subject provides written informed consent. Active duty military will not be specifically recruited for this study. Active duty military members interested in participating will need to obtain supervisor approval. To minimize any possible coercion, supervisors or anyone in the chain of command or in a dominant position will not be permitted to consent the subject.

### 9.3. Eligibility Screening

Each subject must meet all inclusion and no exclusion criteria. The PI or designee will make the final decision of the eligibility. Only eligible subjects will be given the investigational product.

### 9.4. Subject Inclusion Criteria

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 ≥ 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.

### 9.5. Subject Exclusion Criteria

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

### 9.5.1. 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 chemotherapeutics 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 section 8.1).
8. Systemic antimicrobial treatment (i.e., topical treatments are not an exclusion) within 1 week before administration of the first vaccine.

### 9.5.2. 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.

### 9.5.3. 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.

## 9.6. Subject Withdrawal Criteria

Each subject may withdraw consent at any time during the study without penalty. Counseling about the subject's health will be provided if he or she decides to discontinue participation in the study. Medical advice regarding what is in the best interest of the subject will be provided.

The PI may discontinue the 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

### 9.6.1. When and How to Withdraw Subjects

A subject may end his or her participation in the study at any time. If a subject withdraws, the investigator will make a reasonable effort to determine the reason for the withdrawal from the study and to complete termination procedures. Telephone calls, registered letters, and e-mail correspondence are considered reasonable effort. For subjects leaving the study, a targeted examination may be performed if medically indicated and if permitted by the subject.

A subject may be withdrawn for an AE or SAE resulting in a safety concern or for noncompliance with protocol requirements. When a subject withdraws or is withdrawn by the PI due to an AE that results in a safety concern, the sponsor must be notified within 72 hours. Investigators must follow specific policy at each institution regarding the timely reporting of AEs and SAEs to the local IRB (section 11.9.1.2). In all cases, the PI will make a reasonable effort to complete study termination procedures. Subjects who do not receive subsequent vaccine doses but who continue in follow-up for safety and immunogenicity are not considered to be withdrawn from the study.

If a subject meets withdrawal conditions for a concomitant medication violation or noncompliance, this should clearly be stated in the source document and the study termination eCRF.

### 9.6.2. Follow-up for Withdrawn Subjects

Attempts will be made to follow all subjects for safety up to 28 days after receipt of the last vaccine dose. Short-term safety follow-up 7-days post vaccination will also be attempted.

### 9.6.3. Data Collected for Withdrawn Subjects

All data collected up to the time of withdrawal will be reported, unless consent is withdrawn. If the subject proceeds with the specific follow-up procedures for withdrawn subjects, this data will also be collected and reported. The study termination eCRF will be completed with the reason for withdrawal specified.

### 9.6.4. Replacement of Subjects

Alternates recruited for each cohort will be asked to come to the day of the first vaccination for each cohort. If an assigned subject does not present on the first day of vaccination, elects to withdraw, or is found to no longer be eligible, an alternate will be enrolled. Subjects who withdraw or are withdrawn after the first vaccination will not be replaced.

## 10. Treatment of Subjects

If the 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 returns to be enrolled in a subsequent dose group whose screening was performed more than 60 days prior to the vaccination date, he or she will be screened again for eligibility. This screening will include the labs required 7 days prior to vaccination (between Days -14 to -2) as delineated in section 8.1 (Study and Screening Schedule). If the 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.

### 10.1. Dose Administration/Vaccination and Follow-up Periods/Study Visits

#### 10.1.1. Administration of Vaccination

The vaccine or placebo will be administered intramuscularly to alternating deltoid regions, per the Time and Events Schedule (Table 8). The vaccine or placebo will be administered in accordance with formulation and vaccination procedures outlined in the MOP.

#### 10.1.2. Handling of Study Samples

Samples collected under this protocol will be used to conduct protocol-related safety and immunogenicity evaluations. Sera and whole blood will be stored at the WRAIR in quality-controlled environments. Transport and storage of these biological samples will be handled according to appropriate procedures. Any study for the future use of these biological samples will undergo appropriate regulatory review. All subjects will consent for the future use of their specimens.

### 10.2. Concomitant Medications

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.

### 10.3. Procedures for Monitoring Subject Compliance

All vaccines will be administered or witnessed by a study investigator, clinical research coordinator, or designee.

## 11. Laboratory and Clinical Assessment

### 11.1. Blood Sample Collection

Study serology and immunological analyses will be performed from blood samples drawn at several of the follow up clinic appointments. Blood for serologic assays will be obtained per the Time and Events Schedule.

### 11.2. Stool Sample Collection

Stool will be collected from subjects for immunogenicity testing for fecal IgA. Subjects will be provided stool hats to self-collect all stools which will be processed within two hours of receipt in the CTC and within 8 hours of collection by the subject, according to study specific procedures that have been utilized successfully for other enteric disease studies.

### 11.3. Saliva Sample Collection

Collection of saliva samples will be performed utilizing synthetic oral swabs (Oracol Swab; Malvern Medical). The subject will gently rub the sponge portion of the Oracol Swab along their gum line for approximately one minute. Subjects will be instructed not to eat or drink anything, including chewing gum or tobacco, for 10 minutes prior to saliva collection. Subjects will be instructed to avoid drinking alcohol or using mouthwash for 24 hours and to avoid caffeinated beverages for 12 hours prior to collecting the sample. Saliva collection will proceed regardless of subject compliance with these instructions. Noncompliance with these instructions will not be considered a protocol deviation but will be documented.

### 11.4. Study Safety Management

The IRB, research monitor, and PI will review any safety concerns.

Safety monitoring will be conducted throughout the study; therefore, safety concerns will be identified by continuous review of the data by the PI, clinic staff, Research Monitor, and PSRT.

#### 11.4.1. Protocol Safety Review Team

The Protocol Safety Review Team (PSRT), comprised of the Principal Investigator, the PATH Medical Officer, and the Independent Research Monitor will routinely monitor safety throughout the duration of the trial. The PSRT will be chaired by the Independent Research Monitor, and may seek additional independent expert medical opinion as dictated by needs. Emmes will prepare blinded safety reports for review by the PSRT. These reports will provide at a minimum the following information: 1) accrual and participant status data with regard to completion of study vaccinations and study visits; and 2) summaries of solicited and unsolicited adverse events including safety labs. The PSRT safety review will be conducted electronically (or by teleconference when needed). In addition to safety review, the PSRT may elect to discuss trial conduct issues that impact study integrity and participant safety. These may include but are not limited to data quality, critical monitoring findings, study product, research specimens, etc.

Emmes will also notify the PSRT of ad hoc safety reviews whenever it is aware of SUSAR or adverse events that meet pre-specified study pause criteria as per section 8.10.

In addition to routine review of safety information, a central role of the PSRT is the blinded review of safety data for the recommendation of whether to progress to the next dose level in each cohort and to the next cohort. Cumulative safety data will be available continuously for review by PSRT members, and safety reports will be prepared for each PSRT deliberation of dose escalation and progression to subsequent cohorts.

### 11.4.2. Research Monitor

The research monitor will function as an independent safety advocate for subjects per Army Regulation 70-25 and DoD Instruction 3216.02. An independent research monitor is required to review all unanticipated problems involving risk to subjects or others, SAEs, and all subject deaths associated with the protocol and provide an unbiased written report of the event. At a minimum, the research monitor should comment on the outcomes of the event or problem and, in the case of an SAE or death, comment on the relationship to participation in the study. The research monitor should also indicate whether he or she concurs with the details of the report provided by the study investigator. Reports for events determined by either the investigator or research monitor to be possibly or definitely related to participation and reports of events resulting in death should be promptly forwarded to the IRB.

## 11.5. Specification of Safety Endpoints

### 11.5.1. Local and Systemic Reactions

Signs and symptoms will be specifically surveyed at each visit as well as by patient self-report using a symptom diary. Symptoms that will be specifically solicited are listed in section 11.6.2. Additionally, we will record all unsolicited symptoms. These local reactions will be coded for severity based on the subject and investigator severity grading scale.

### 11.5.2. Vital Signs

Vital signs (temperature, blood pressure, and heart rate) will be obtained at each study visit (Table 9).

**Table 9: Reference Ranges and Adverse Event Coding for Vital Sign Parameters**

| Vital Signs | 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)<br>(°F) | 38.0–38.4<br>100.4–101.1 | 38.5–38.9<br>101.2–102.0 | 39.0–40<br>102.1–104 | > 40<br>> 104 |
| **Blood Pressure** | | | | |

| Vital Signs | Mild (Grade 1) | Moderate (Grade 2) | Severe (Grade 3) | Potentially Life Threatening (Grade 4) |
|---|---|---|---|---|
| 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[b] (systolic, mm Hg) | 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.

[c] If a subject visits 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 severity of the AE will be assessed according to the subject's clinical signs and symptoms.

### 11.5.3. Physical Examination

A complete physical exam will be conducted during the screening visit as part of the screening process. Subsequent focused clinical examinations will occur at each study visit with specific attention to the identification of local, systemic, or other adverse reactions.

### 11.5.4. Clinical Laboratory Assessments

Venous blood samples will be collected for chemistries and hematology. Hematology and chemistry analyses (BUN, creatinine, AST, ALT, electrolytes, and glucose) will occur per the Time and Events Schedule. Hematology and chemistry analyses will be performed by a commercial laboratory (Quest Diagnostics, Incorporated). Additional specimens may be collected to confirm and evaluate any abnormal values.

The clinical toxicity grading scale that will be used as a guideline is based on the Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. Final grading determination will be made by the PI based on normal lab values for the specific lab and clinical symptoms. If any additional safety labs are performed, the FDA Guidance for Industry will be utilized.

#### 11.5.4.1. Hematology

The following hematology parameters (Table 10) will be assessed:

- White blood cell count (and differential)
- Red blood cell count
- Hematocrit
- Platelet count
- Hemoglobin

**Table 10: Reference Ranges and Adverse Event Coding for Clinical Hematology Parameters**

| Test | 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<br>F: LLN = 11.7 | M: 12.5-13.1<br>F: 11.0-11.6 | M: 10.5-12.4<br>F: 9.5-10.9 | M: 8.5-10.4<br>F: 8.0-9.4 | M: <8.5<br>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.

### 11.5.4.2. Blood Chemistry

The following clinical chemistry parameters listed in Table 11 will be assessed:

**Table 11: Reference Ranges and Adverse Event Coding for Blood Chemistry Parameters**

| Test | 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<br>F: 10-30 U/L | M: 41-100<br>F: 31-75 | M: 101-200<br>F: 76-150 | M: 201-400<br>F: 151-300 | M: > 400<br>F: > 300 |
| SGPT/ALT (elevation) | M: 9-60 U/L<br>F: 6-40 U/L | M: 61-150<br>F: 41-100 | M: 151-300<br>F: 101-200 | M: 301-600<br>F: 201-400 | M: > 600<br>F: > 400 |
| BUN (elevation) | 7-25 | 26-28 | 29-31 | > 31 | Requires dialysis |
| Creatinine (elevation) | M: 0.7-1.4<br>F: 0.5-1.1 | M: 1.5-1.7<br>F: 1.2-1.7 | M: 1.8-2.0<br>F: 1.8-2.0 | M: 2.1-2.5<br>F: 2.1-2.5 | M: >2.5<br>F: >2.5 or requires dialysis |

### 11.5.4.3. C-Reactive Protein

C-Reactive Protein (CRP) will be assessed on study days 1, 2, and 8. CRP assessment is for exploratory purposes only (immunologic response and safety endpoints) and will not be used as an eligibility criterion or safety signal. There are few published reports addressing healthy subjects' CRP responses after vaccination. Both the correlation of CRP to immune responses and to systemic AEs (malaise, fever) has been performed in these trials [21-24].

In this trial, collection of CRP will be limited to baseline and two time points after initial vaccination.

### 11.5.4.4. Virus Testing

Testing for evidence of HIV-1/HIV-2, HCV, and HBV infections will be obtained during the screening process as delineated in the time and events schedule (Table 8) and no earlier than 14 days before initial vaccination. Evidence of infection will make a subject ineligible. Additional diagnostic testing will not be performed as part of this study.

#### 11.5.4.5. HLA-B27 Screen

Serologic evidence of HLA-B27 will be obtained during the screening process (Days -60 to -9). Evidence of HLA-B27 positivity will make a subject ineligible.

#### 11.5.4.6. Drug Screen

No drug screening is planned for this study described herein.

#### 11.5.4.7. Pregnancy Screen

A urine sample for pregnancy testing (female subjects of childbearing capacity) will be collected at the screening visit. Pregnancy tests will also be obtained on each day of vaccination and at the final in-clinic study visit. A positive pregnancy test prior to vaccination will result in no additional study vaccinations being provided. Any subjects who become pregnant during the study will be removed from receiving further treatment with Invaplex$_{AR\text{-}DETOX}$ and followed until the end of their pregnancy. Procedures to be followed in the event a study participant becomes pregnant during the study period are outlined in 11.9.2.1.

### 11.6. IND Safety Reporting

The following terms, as defined by 21 Code of Federal Regulations (CFR) 312.32, apply to IND safety reporting.

#### 11.6.1. Adverse Event or Suspected Adverse Reaction

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

### 11.6.2. Solicited Adverse Events

A solicited AE is a predetermined event, identified in the Investigator's Brochure, which may reflect safety concerns related to the investigational product. The solicited AEs for this study include:

- Site pain
- Site tenderness
- Swelling
- Induration (determined by investigator exam)
- Site redness
- Pruritus
- Fever
- Nausea
- Vomiting
- Abdominal pain
- Diarrhea (loose stools)
- Appetite change
- Fatigue
- Headache
- Myalgias (general pain or soreness in muscles)
- Arthralgias (general pain in joints)
- Malaise

### 11.6.3. Serious Adverse Event 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.

### 11.6.4. 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.

### 11.6.5. Other Adverse Event

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.

## 11.7. 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.

## 11.8. Recording Adverse Events

### 11.8.1. Methods/Timing for Assessing, Recording, and Analyzing Safety Endpoints

AEs, solicited AEs, and SAEs will be assessed at all study visits, documented in the source records, and recorded on the eCRFs using accepted medical terms and/or the diagnoses that accurately characterize the event. When a diagnosis is known, the AE term recorded on the eCRF will be the diagnosis rather than a constellation of symptoms. The investigator will assess all AEs for seriousness, relationship to the investigational product, severity, and other possible etiologies. When an event has not resolved by the prescribed reporting period, it will be documented on the AE eCRF as "unknown."

The time frame for the collection of AEs and SAEs begins at the first administration of investigational product through 28 days after the last dose of investigational product is administered. Additionally, subjects will be contacted by telephone approximately 6 months after the final vaccination to assess for any new-onset SAEs since participation in the trial.

### 11.8.2. Duration of Follow-up of Subjects After Adverse Events

The PI and/or subinvestigators will follow SAEs to resolution, even if this extends beyond the prescribed reporting period. Resolution is the return to baseline status or stabilization of the condition with the probability that it will become chronic. SAE outcomes will be reported to the sponsor using the supplemental Serious Adverse Event Report Form.

Investigators are not obligated to actively seek SAEs in former subjects; however, if an SAE, considered to be related to the investigational product is brought to the attention of the investigator at any time following completion of the study, the event will be reported to the sponsor as defined in section 11.9.1.1.

### 11.8.3. Severity Assessment

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 section 11.5.2 for vital signs and section 11.5.4 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<br>Moderate level of discomfort |
| **Severe** | Grade 3 | Unable to perform routine activities<br>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.

## 11.9. Reporting Adverse Events

The PI will report all AEs to the sponsor and the local IRB in the appropriate safety, annual, and/or final reports. The investigative team will prepare annual reports and final reports for submission to the FDA.

### 11.9.1. Reporting Serious and Adverse Events

Contact information for reporting SAEs and unanticipated problems is provided in Table 12.

#### 11.9.1.1. Reporting to the Sponsor

All SAEs and unexpected AEs must be reported promptly (within 72 hours) to the sponsor, whether or not the event is considered related to the study product. Further, the investigator should comply with relevant study-site SOPs on reporting SAEs.

The information that the investigator will provide to the sponsor is specified in Table 13. The sponsor may request additional information for purposes of the study.

**Table 12: Study Contacts for Reporting Serious Adverse Events**

| | |
|---|---|
| **Sponsor's Medical Officer** | Rahsan Erdem, MD<br>PATH<br>455 Massachusetts Ave NW<br>Suite 1000<br>Washington, DC 20001<br>Telephone: 202-540-4546<br>Fax: 202-457-1466<br>E-mail: rerdem@path.org |
| **Institutional Review Board** | Naval Medical Research Center<br>Research Services Directorate Office of Research Administration Code 025, Building 500, Room 004<br>Silver Spring, MD Telephone: 301-319-7276<br>Fax: 301-319-7277 |

| | |
|---|---|
| **Research Monitor** | Michael Koren, MD<br>WRAIR<br>Telephone: 301-319-9904<br>E-mail: michael.a.koren2.mil@mail.mil |
| **Research Monitor (alternate)** | James Moon, MD<br>WRAIR<br>Telephone: 301-319-9176<br>E-mail: james.e.moon.mil@mail.mil |

**Table 13: SAE Information to Be Reported to the Sponsor**

| Notification Method | Information to Be Provided |
|---|---|
| **E-mail or Telephone (within 72 hours)** | IND number, sponsor study number, name of the investigational product, and investigator name and contact number |
| | Subject identification number |
| | SAE, onset date, date of investigational product administration, severity, relationship, and subject's current status |
| **AND** | |
| **E-mail or Fax** | Cover sheet or letter |
| | Adverse event case report form |
| | Supplemental SAE report forms |
| | Concomitant medication case report form or a list of concomitant medications |
| | Medical record progress notes including pertinent laboratory/diagnostic test results |
| NOTE: When submitting SAE reports via e-mail, the subject line of each e-mail notification will read as follows: **SAFETY REPORT – IND # _____, Sponsor Study #_____, Subject# _____, Event Term: _____** | |

To comply with regulations mandating sponsor notification of specified SAEs to the FDA within 7 calendar days, investigators must submit additional information as soon as it is available. The sponsor will report unexpected SAEs associated with the use of the drug to the FDA as specified in 21 CFR 312.32 (c).

Investigators must follow all relevant regulatory requirements as well as specific policy at each institution regarding the timely reporting of SAEs to the NMRC IRB, and the research monitor.

### 11.9.1.2. Reporting to the IRB

Unanticipated problems involving risk to subjects or others, SAEs related to participation in the study, and all subject deaths should be promptly reported by phone, e-mail, or fax to the local IRB.

It is the NMRC's policy to report to the IRB within 24 hours after determination of the event. A formal written report is due within 5 business days of AE reporting.

The following AEs should be considered as unanticipated problems that must be reported to the IRB:

- A single occurrence of a serious, unexpected, and uncommon event that is strongly associated with exposure to the investigational product.

- A single or small number of a serious, unexpected event that is not commonly associated with exposure to the investigational product but is uncommon in the study population.
- Multiple occurrences of an AE that, based on aggregate analysis, is determined to be an unanticipated problem. There should be a determination that the series of AEs represents a signal that the AEs were not just isolated occurrences and involve risk to human subjects.
- An AE that is described in the Investigator's Brochure, protocol, or informed consent documents but occurs at a specificity or severity that is inconsistent with prior observations.
- An SAE that is described in the Investigator's Brochure, protocol, or informed consent documents but for which there is a clinically significant increase in the expected rate of occurrence.
- Any other AE or safety finding, including those based on animal or epidemiologic data that would cause the sponsor to modify the Investigator's Brochure, protocol, or informed consent documents or would prompt other action by the IRB to ensure the protection of human subjects.

All SAEs will be reported to the Western Institutional Review Board (WIRB) by the sponsor according to the WIRB guidelines and using the WIRB Promptly Reportable Information Form.

WIRB Phone: 800-562-4789, Fax: 360-252-2498

PATH is required to report SAEs or other events that fit the following criteria within 5 working days of the time of becoming aware of them:

- New or increased risk
- Protocol deviation that harmed a subject or placed subject at risk of harm
- Protocol deviation made without prior IRB approval to eliminate an immediate hazard to a subject
- Audit, inspection, or inquiry by a federal agency
- Written reports of federal agencies (e.g., FDA Form 483)
- Allegation of Noncompliance or Finding of Noncompliance
- Breach of confidentiality
- Unresolved subject complaint
- Suspension of premature termination by the sponsor, investigator, or institution
- Incarceration of a subject in a research study not approved to involve prisoners
- Adverse events or IND safety reports that require a change to the protocol or consent
- State medical board actions
- Unanticipated adverse device effect
- Information where the sponsor requires prompt reporting to the IRB

### 11.9.2. Reporting Additional Immediately Reportable Events to the Sponsor and the NMRC IRB

#### 11.9.2.1. Pregnancy

Each pregnancy must be reported *immediately* (within 72 hours of identification) by e-mail or fax to the sponsor and the NMRC IRB.

Subjects who become pregnant after receiving the first dose of vaccine or up to 3 months after the last vaccination will be followed to term, and information will be gathered for outcome, date of delivery, and health status of the mother and child including the child's gender, length, and weight. Complications and/or abnormalities should be reported including any premature terminations. A pregnancy is reported as an AE or SAE only when there is suspicion that the investigational product may have interfered with the effectiveness of contraception or there was a serious complication in the pregnancy including a spontaneous abortion or an elective termination for medical rationale. Principal investigator will notify study monitor, the local IRB, the research monitor and the sponsor for subjects that become pregnant during the study. The subject will be asked to provide serial follow-ups on the status of her pregnancy as well as provide health information on her infant following delivery. The subject will be asked to provide copies of clinic visits only if needed. The Subjects will receive no further vaccinations but will continue safety monitoring and all follow up visits as noted in the study schedule.

#### 11.9.2.2. Adverse Event-related Withdrawal of Consent

Any AE-related withdrawal of consent due to a safety concern during the study must be reported *immediately* (within 72 hours of identification) by e-mail or fax to the sponsor and the NMRC IRB.

#### 11.9.2.3. Pending Inspections/Issuance of Reports

The knowledge of any pending compliance inspection/visit by the FDA, Office for Human Research Protections (Department of Health and Human Services), or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, Form FDA 483, warning letters, or actions taken by any regulatory agency including legal or medical actions, and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to the NMRC IRB and the sponsor.

### 11.9.3. IND Annual Report to the FDA

The PI will be responsible for the preparation of a detailed annual synopsis of clinical activity, including AEs, for submission to the sponsor. Each annual report will summarize IND activity for 1 year beginning approximately 3 months before the IND FDA anniversary date. The sponsor will notify the PI of the due date with sufficient time for the PI to assemble the required information.

### 11.9.4. Final Report

A final study report will be prepared in accordance with Guidance for Industry: Submission of Abbreviated Reports and Synopses in Support of Marketing Applications and ICH E3 Guideline

Structure and Content of Clinical Study Reports and provided to the sponsor for review and approval. The sponsor will use this report to prepare the submission to the FDA.

## 12. Statistics

Detailed statistical procedures, listings, table shells, and figures will be provided in a separate statistical analysis plan (SAP) written shortly after protocol approval but before any subject enrollment. The SAP will be finalized before study closeout and database lock. The following key statistical components will be considered, and a detailed description will be documented in the SAP:

- Primary and secondary endpoints and how they will be measured,
- Statistical methods and tests that will be used to analyze the endpoints,
- Strategy that will be used if the statistical test assumptions are not satisfied,
- Indication of whether the comparisons will be one-tailed or two-tailed (with justification of the choice) and the level of significance to be used,
- Identification of whether any adjustments to the significance level or the overall
- p value will be made to account for any planned or unplanned subgroup analyses or multiple testing,
- Specification of potential adjusted analyses and a statement of which covariates or factors will be included,
- Planned exploratory analyses and justification with their importance, and
- Any subgroup effects with biological justification and support from within and outside the study.

### 12.1. Description of Statistical Methods

#### 12.1.1. Analysis Addressing the Primary Study Objective (Safety Analysis)

The primary objective of this study is safety of the Invaplex$_{AR\text{-}DETOX}$ vaccination. All immunized subjects will be included in the safety analysis. AE data will be listed individually and summarized by system organ class and preferred terms within a system organ class for each treatment group. Serious and/or unexpected AEs will also be discussed on a case-by-case basis. For the tabulation of AEs by system organ class, a subject will be counted only once in a given system organ class. For example, a subject reporting nausea and diarrhea will be reported as one subject, but the symptoms will be listed as two separate AEs within the class. Therefore, the total number of AEs reported within a system organ class may exceed the number of subjects within the system organ class reporting AEs.

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. Summary tables will be created to indicate the number of subjects who experienced events. Vaccine-related events (definitely, probably, or possibly related) will be tabulated by study group. In addition, tables will be prepared to list each adverse event, the number of subjects in each treatment group

who experienced an event at least once, and the rate of subjects with adverse event(s). Adverse events will be divided into defined severity grades (mild, moderate, severe, potentially life-threatening). The tables will also divide the adverse events by relationship to the investigational product if applicable. All immunized subjects will be included in the safety analysis.

Clinical and laboratory data regarding safety, which include vital signs and safety laboratory measures, will be included in the primary analysis. Changes in pulse rate, systolic and diastolic blood pressure, weight, and respiratory rate will be compared within each group and among groups using analysis of variance procedures. For hematology and serum chemistry tests, the mean, mean change, median, median change, and range of all values for each test for each treatment group at baseline and for the final "on therapy" values will be tabulated. The tables will also divide the adverse events into those considered at least possibly related to the vaccine and those considered not related.

If serious adverse events occur, they will be listed separately delineating outcome, treatment, concomitant medication, relevant medical history, and laboratory measurements.

### 12.1.2. Analysis Addressing the Secondary Study Objective

For immunology responses, both qualitative (responder rates) and quantitative (e.g., $\log_{10}$ transformed values) results will be analyzed. Geometric mean titers will be calculated for serological responses along with their 95% confidence intervals (or standard deviation). If appropriate, between groups comparisons will be examined with nonparametric median tests (Kruskal-Wallis for continuous data and Fisher's exact test for categorical data) unless assumptions are fulfilled for Student's t or $\chi^2$.

Additional comparisons may be made using repeated measures analysis of variance with study group as the between subject factor and sample collection time-points as the repeated factor. Only subjects who receive at least 2 vaccine doses will be included in the immunology analysis. All statistical tests will be interpreted in a two-tailed fashion using an $\alpha$=0.05 to represent statistical significance.

### 12.1.3. Clinical Laboratory Data Analyses

Hematology and serum chemistry test values will be analyzed and evaluated. These evaluations will be reviewed by the PI or research monitor to evaluate whether any significant trends in laboratory values occurred.

## 12.2. Planned Enrollment and Reason for Sample Size

A total of 60 subjects are planned for this study. The sample size for this study is limited by the early stage (Phase 1) of the product concept/testing and is 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 our estimate for AEs is limited. For example, using binomial probability formulae for no observed adverse events within the 16 subjects yields a 95% confidence interval of 0-21%. Follow-on studies evaluating seemingly safe and immunogenic doses will be required with larger numbers of volunteers to better define the safety profile.

### 12.3. Accounting for Missing, Unused, and Spurious Data

Non-analyzable data will be documented in the deviations.

### 12.4. Procedures for Reporting Deviations from the Original Statistical Plan

Any deviation(s) from the original statistical plan as indicated in the protocol will be described in an amendment to the protocol and the SAP. Deviations from the SAP will be documented in accordance with NMRC SOPs.

### 12.5. Selection of Subjects to Be Included in Analyses

All subjects who receive at least one dose of the investigational product will be included in the safety analyses. All subjects who receive at least two doses of the investigational product will be included in the immunological analyses.

## 13. Direct Access to Source Data/Documents

Subjects will be identified on eCRFs by a unique subject identification number and on source documents by name and date of birth. No personal identifier will be used in any publication or communication used to support this research study. The subject identification number will be used if it becomes necessary to identify data specific to a single subject. Representatives of the sponsor, the local IRB, and the FDA are eligible to review medical and research records related to this study as a part of their responsibility to protect human subjects in clinical research. Personal identifiers will be removed from photocopied medical and research records.

### 13.1. Study Monitoring

Study monitoring will be the responsibility of the clinical monitoring team. Upon successful approval of the protocol and establishment of the regulatory file, the clinical monitor will establish a clinical monitoring plan. To ensure that the investigator and the study staff understand and accept their defined responsibilities, the clinical monitor will maintain regular correspondence with the site and may be present during the course of the study to verify the acceptability of the facilities, compliance with the investigational plan and relevant regulations, and the maintenance of complete records. The clinical monitors may observe study procedures as needed to ensure subject safety and rights are protected and verify compliance with the protocol.

Monitoring visits will be scheduled to take place at the initiation of the study, during the study at appropriate intervals, and after the last subject has completed the study. A report of monitoring observations will be provided to the PI (for corrective actions), the sponsor, and the product manager.

### 13.2. Audits and Inspections

Authorized representatives of the sponsor, the FDA, the Independent Ethics Committee or IRB, and the Department of the Navy Human Research Protections Program may visit the site to perform audits or inspections, including source data verification. The purpose of the audit or inspection is to systematically and independently examine all study-related activities and documents to determine whether these activities were conducted and data were recorded,

analyzed, and accurately reported according to the protocol, ICH GCP, and any applicable regulatory requirements.

The investigator should contact the sponsor and the NMRC IRB immediately (within 24 hours) if contacted by a regulatory agency about an inspection.

### 13.3. Institutional Review Boards

The PI must obtain IRB approval for the study. Initial IRB approval and all materials approved by the IRB for this study, including the subject consent form and recruitment materials, must be maintained by the investigator and made available for inspection.

The PI will be responsible for preparing and submitting continuing review reports per institution and IRB policies. The PI or a designee will submit the approved continuing review reports and the local IRB approval notifications to HRPO (if applicable) as soon as the documents are available.

WIRB will provide sponsor level review for PATH Vaccine Solutions (PVS) for this study. PVS will submit the AE/SAE to WIRB only if it is considered unanticipated and related to study product(s) according to the WIRB defined procedures.

## 14. Quality Control and Quality Assurance

During the study, the investigator will maintain complete and accurate documentation for the study, including records detailing the progress of the study for each subject; laboratory reports; eCRFs; a signed informed consent form for each study subject; drug disposition records; correspondence with the IRB, the study monitor and the sponsor; AE reports; and information regarding subject discontinuation and completion of the study.

All required study data will be recorded clearly and accurately by authorized study personnel in the eCRFs. Only designated study-site personnel shall record or change data in the eCRFs. During the study, the investigator will be responsible for ensuring the quality of data recorded in the eCRFs as per written eCRF completion guidelines.

To ensure compliance with GCP and all applicable regulatory requirements, the sponsor may conduct quality assurance audits.

Auditing of the clinical trial may be conducted at any time during the study to ensure continued compliance with regulations, policies, and procedures. Auditing will be undertaken, as needed, by independent personnel designated by the sponsor. Audit findings will be documented in a formal audit report that will detail the conduct of the audit and summarize the observations noted.

## 15. Ethics

### 15.1. Ethics Review

The study is based on adequately performed laboratory and animal experimentation and will be conducted under a protocol reviewed by the NMRC IRB. The study is to be conducted by scientifically and medically qualified persons. The rights and welfare of the subjects will be

respected, the physicians conducting the study will ensure that the hazards do not outweigh the potential benefits, the results to be reported will be accurate, subjects will give their informed consent and will be competent to do so and not under duress, and all study staff will comply with the ethical principles in 21 CFR Part 50 and the Belmont Principles.

### 15.1.1. Review/Approval of Study Protocol

Before a clinical study can be initiated, the study protocol and other required documents will be submitted to the following for review and/or approval. Additionally, the protocol may be presented to the USAMRMC Commanding General prior to study initiation. The timing of that presentation will vary depending on other extraneous factors.

- NMRC Scientific Review Board
- PATH
- NMRC IRB
- Commander, WRAIR
- FDA
- WIRB (sponsor level review)

Enrollment in this protocol may not begin until all approvals have been obtained and the sponsor has authorized study start.

The investigator will not take actions that require protocol amendment for any reason without obtaining appropriate review/approval, except in cases of medical emergencies.

### 15.1.2. Protocol Modifications

All modifications to the protocol and supporting documents (informed consent, MOP, SOPs, recruitment materials, etc.) must be reviewed and approved prior to implementation. Any protocol amendment will be agreed upon by the sponsor prior to submission to the local IRB and prior to implementation of said change or modification. The informed consent document must be revised to concur with any amendment as appropriate and must also be reviewed and approved with the amendment. Any subject already enrolled in the study will be informed about the revision and asked to sign the revised informed consent document if the modification directly affects the individual's participation in the study. A copy of the revised, signed, and dated informed consent document will be given to the subject. All original versions of the informed consent document will be retained in the protocol regulatory file, and a copy will be retained in the clinic medical record.

Any modification that could potentially increase risk to subjects must be submitted to the NMRC IRB, WIRB, and the FDA for approval prior to implementation. Documentation that the local IRB reviewed and approved the modifications also will be submitted. All other amendments will also be submitted to the NMRC IRB for inclusion in the HRPP study file.

### 15.1.3. Protocol Deviation Procedures

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 MOP. Deviations will be reported annually in the continuing review report to the IRB and HRPO, if appropriate. The PI or subinvestigator 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.

## 15.2. Ethical Conduct of the Study

This study will be conducted in accordance with all applicable federal and DoD human research protections requirements and the Belmont Principles of respect for persons, beneficence, and justice.

The procedures set out in this study are designed to ensure that the sponsor and all study personnel abide by the principles of the ICH GCP Guideline and the CFR. The PI confirms this by signing this study protocol and Form FDA 1572.

### 15.2.1. Confidentiality

In this research, the subject's health information will be collected and used to conduct the study; monitor the subject's health status; measure effects of the investigational product; determine research results; and possibly to develop new tests, procedures, and commercial products. Health information is used to report results of research to the sponsor and federal regulators and may be reviewed during study audits for compliance with study plans, regulations, and research policies. After the study ends, each subject has the right to see and receive a copy of his or her information.

The sponsor, the DoD, and the FDA are eligible to photocopy and review records related to this protocol as a part of their responsibility to protect the participants of this treatment protocol. No personal identifier will be used in any publication or communication used to support this research study. The subject's identification number will be used in the event it becomes necessary to identify data specific to a single subject.

All study procedures will be conducted per GCP guidelines, and every effort will be made to protect volunteer privacy and confidentiality to the extent possible.

All study-related information will be stored securely at the study site or at a designated, secure off-site location. While not in use and under immediate control of study staff, all volunteer information will be stored in locked areas with access limited to study staff. Data collection, process and administrative forms, laboratory specimens, and other reports will be identified exclusively by a coded number to maintain volunteer confidentiality. All local databases will be secured with password-protected access systems. Volunteers' study information will not be released without their written permission, except as necessary for monitoring or compliance with legal or regulatory requirements. Additionally, the study team does not foresee any commercial value for the samples collected in this study.

Medical records containing identifying information may be made available for review when the sponsor or an authorized regulatory agency monitors the study. Direct access may include

examining, analyzing, verifying, and reproducing any records and reports that are important to the evaluation of the study.

### 15.2.2. Compensation for Participation

Compensation will occur at the time of each designated visit. Compensation will be provided only for completed study procedures designated for compensatory payment. Subjects will only be eligible for compensation outlined in the informed consent documents at the time their consent is obtained. If a subject is eligible to participate in the investigation protocol after screening, he or she will receive the following compensation:

**Civilian/off-duty military compensation:**

- Screening: $25
- Day -7: $100
- Day 1: $150
- Day 2: $100
- Day 8: $100 + $20 (if subjects return and complete symptom diary)
- Day 22: $150
- Day 23: $100
- Day 29: $100 + $20 (if subjects return and complete symptom diary)
- Day 43: $150
- Day 44:$100
- Day 50: $100 +$20 (if subjects return and complete symptom diary)
- Day 57: $100
- Day 71: $160

Total: $1,495 (maximum if subjects return all symptom diaries)

If subjects do not complete the study, their compensation will be less in proportion to the amount they did not complete.

Visits that are not specifically planned in the protocol, such as may be required to repeat labs to verify/clarify results or labs drawn to better evaluate abnormal lab values or adverse events may be compensated at the discretion of the PI relative to the severity of the event and scope of evaluation.

Subjects asked to come to the clinic as an alternate on the first vaccination day of a cohort may be compensated $50 if they are not enrolled in the cohort.

**Federal Employee/on-duty military compensation:**

By regulation, active duty personnel and federal employees can be compensated only for visits in which blood draws occur, and then only $50 per visit, unless the visits occur during off-duty hours or when they are on leave. If the volunteer is off-duty or on leave, he or she will be paid

the same as non-military/non-federal personnel. The total amount of compensation may vary depending on the number of visits completed.

- Screening: $25
- Day -7: $50
- Day 1: $50
- Day 2: $50
- Day 8: $50
- Day 22: $50
- Day 23: $0
- Day 29: $50
- Day 43: $50
- Day 44: $0
- Day 50: $50
- Day 57: $50
- Day 71: $50

Total: $525

### 15.2.3. Redress of Research-Related Injury

All study-related medical care will be provided without cost. Should a subject be injured as a direct result of participating in this research project, he or she will be provided medical care by the staff at the Walter Reed National Military Medical Center (or other military-affiliated medical center), at no cost to the subject, for that injury. Subjects will not receive any injury or disability compensation, only medical care. Subjects will not be compensated for care if they choose to seek care from their own physicians.

All nonexempt research involving human subjects shall, at a minimum, meet the requirement of 32 CFR 219.116(a)(6).

If a subject is injured because of participation in this research and is a DoD health care beneficiary (e.g., active duty in the military, military spouse or dependent), the subject is entitled to medical care for that injury within the DoD health care system as long as the subject remains a DoD health care beneficiary. This care includes, but is not limited to, free medical care at Army hospitals or clinics.

If a subject is injured because of participation in this research and is not a DoD health care beneficiary, the subject is entitled to free medical care for that injury at an Army hospital or clinic. It cannot be determined in advance which Army hospital or clinic will provide care. If the subject receives care for research-related injuries outside of an Army hospital or clinic, the subject or the subject's medical insurance will be responsible for medical expenses.

For all subjects: Transportation to and from Army hospitals or clinics will not be provided. No reimbursement is available if the subject incurs long-term medical expenses to treat research-related injuries. No compensation is available for research-related injuries or related disability. The subject is not waiving any legal rights. The subject should contact the PI if the subject believes he or she has sustained a research-related injury. The subject should contact the PI for any questions.

Requests for other benefits, such as compensation for lost time from work, are processed independently of this protocol. Military members retain the right to pursue military disability benefits, and federal civilian employees retain the right to pursue relief through established workers' compensation processes, but neither military disability benefits nor workers' compensation benefits are guaranteed. The right of other parties to seek redress against the US Government is limited to that set forth by existing agency regulations and the Federal Tort Claims Act. The subject should understand that this does not constitute a waiver or release of legal rights. This issue is addressed in the informed consent and will be discussed with the subject by the investigator or designee before the subject signs the informed consent to participate in the study.

## 15.3. Written Informed Consent

The informed consent process and document(s) will be reviewed and approved by the IRB and sponsor prior to initiation of the study. The consent document(s) contains a full explanation of the possible risks, advantages, and alternate treatment options, and availability of treatment in the case of injury, in accordance with 21 CFR 50. The consent document indicates that by signature, the subject, or where appropriate, legal guardian, permits access to relevant research records by the sponsor and by representatives of the FDA. The sponsor will submit a copy of the initial IRB- and sponsor- approved consent form to the FDA and will maintain copies of revised consent documents that have been reviewed and approved by the IRB/ethics committee.

A written informed consent document, in compliance with 21 CFR Part 50, 32 CFR Part 219 and the Belmont Principles will be signed by the subject before any study-related procedures are initiated for that subject. This consent document must be retained by the investigator as part of the study records. The investigators or their designees will present the protocol in lay terms to individual subjects. Questions on the purpose of the protocol, protocol procedures, and risks to the subjects will then be solicited. Any question that cannot be answered will be referred to the PI. The subject will be allowed to take the consent document home to consider and discuss it with others and can return to the CTC later to sign it. The subject should understand that the study product is an investigational drug and is not licensed by the FDA for commercial use but is permitted to be used in this clinical research. Informed consent includes the principle that it is critical the subject be informed about the principal potential risks and benefits. This information will allow the subject to make a personal risk versus benefit decision and understand the following:

- Participation is entirely voluntary,
- Subjects may withdraw from participation at any time,
- Refusal to participate involves no penalty, and

- The individual is free to ask any questions that will allow him or her to understand the nature of the protocol.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by US law.

Should the protocol be modified, the subject consent document must be revised to reflect the changes to the protocol. If a previously enrolled subject is directly affected by the change, the subject will receive a copy of the revised informed consent document. The approved revision will be read, signed, and dated by the subject.

Military personnel may be recruited by CTC personnel. No recruitment of military personnel will occur in the presence of his or her supervisor. There is no benefit to military personnel for participating in this study. There will be no coercion or disciplinary actions for not participating or withdrawing if enrolled.

## 16. Data Handling and Recordkeeping

The primary source document for this study will be the subject's research record. If separate medical records are maintained by the investigator(s), the medical record and the research records will be considered the source documents for the purposes of auditing the study. The source documents will be retained at the site.

For this study, an EDC database system will be used for the collection of study data in an electronic format. The EDC database system will be designed based on the protocol requirements, the approved eCRF layouts and specifications, and in accordance with 21 CFR Part 11. The eCRF layouts and specifications define and identify the applicable source data that will be collected and captured into the EDC database system. The applicable source data will be electronically transcribed by the site designee onto the eCRF (data entry screens) in the EDC database system. The investigator is ultimately responsible for the accuracy of data transcribed on the eCRF. Data monitoring and management will be performed in the EDC database system by the study monitor and the designated data management group.

A detailed data management plan will be written and approved by the study team and the PI prior to study start, with approval by the sponsor. All updates to the data management plan must be approved before study closeout and database lock.

### 16.1. Inspection of Records

The sponsor or designee will be allowed to conduct site visits to the investigation facilities for the purpose of monitoring any aspect of the study. The investigator agrees to allow the monitor to inspect the drug storage area, investigational product stocks, drug accountability records, subject charts, study source documents, and other records relative to study conduct.

Subjects' health information is used to report results of research to the sponsor and federal regulators and may be reviewed during study audits for compliance with study plans, regulations, and research policies. The consent document indicates that, by signature, the subject permits access to relevant research records by the sponsor and by representatives of the FDA.

The investigator must be aware that representatives from regulatory authorities or the IRB may wish to inspect the eCRFs and associated study records. The investigator will notify the sponsor within 24 hours following contact by a regulatory agency. The investigator and study coordinator must make the relevant records available for inspection and will be available to respond to reasonable requests and audit queries made by authorized representatives of regulatory agencies. The investigator will provide the sponsor with copies of all correspondence that may affect the review of the current study or his or her qualification as an investigator in clinical studies conducted by the sponsor. The sponsor will provide needed assistance in responding to regulatory audits or correspondence.

## 16.2. Retention of Records

The PI must maintain all documentation relating to the study for a period of 2 years after the last marketing application approval or, if not approved, for 2 years following the discontinuance of the investigational product for investigation. If it becomes necessary for the sponsor, their designee, or the FDA to review any documentation relating to the study, the investigator must permit access to such records.

Completed, monitored records will be stored in a secure location.

The PI will be responsible for retaining sufficient information about each subject, that is, name, address, telephone number, social security number, and subject identifier in the study, so that the sponsor, the local IRB, the FDA, employees of USAMRMC, or other regulatory authorities may have access to this information should the need arise.

It is the policy of USAMRMC that data sheets are to be completed for all subjects participating in research (Form 60-R, Volunteer Registry Data Sheet). The data sheets will be entered into this Command's Volunteer Registry Database. The information to be entered into this confidential database includes the subject's name, address, and social security number; study title; and dates of participation. The intent of this database is twofold: first, to readily answer questions concerning an individual's participation in research sponsored by USAMRMC; and second, to ensure that USAMRMC can exercise its obligation to ensure research subjects are adequately warned (duty to warn) of risks and provided new information as it becomes available. The information will be stored at USAMRMC for a minimum of 75 years. The Volunteer Registry Database is a separate entity and is not linked to the study database.

# 17. Publication Policy

All data collected during this study will be used to support this IND. All publications and presentations are governed by the standards and norms detailed in NAVMEDRSCHCENINST 5721.1. All authors will submit the proposed publication/presentation at least 30 days prior to the submission date. Prior to submission, the directorate will conduct a substantive scientific and professional review. The document is routed to the Office of Research Administration (ORA) for review and routing for Command review and approval, ultimately by the NMRC Public Affairs Officer (PAO). Once it is cleared at NMRC, it may be forwarded to BUMED through NMSC, if appropriate. Prior to publication, an author must have a completed Publication Clearance Request Submission Form with signatures from all approving and reviewing authorities.

## 18. List of References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4. **Riddle, M.S., et al., *Incidence, etiology, and impact of diarrhea among long-term travelers (US military and similar populations): a systematic review.* Am J Trop Med Hyg, 2006. 74(5): p. 891-900.**
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7. **Porter, C.K., et al., *The Shigella human challenge model.* Epidemiol Infect, 2013. 141(2): p. 223-32.**
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