Dose-Finding Study of Lyophilized *Shigella sonnei* 53G (Lot 1794) Challenge Strain

IND Sponsor: PATH

Study Number: WRAIR #2286

Manufacturer: Walter Reed Army Institute of Research

Version Number: 7.0

April 14, 2017

STATEMENT OF COMPLIANCE

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

- US CFR applicable to clinical studies (45 CFR 46; a-d 21 CFR including part 50 and 56 concerning informed consent and institutional review board [IRB] regulations, 21 CFR 11 concerning electronic records and 21 CFR 312 for investigational new drug [IND])
- International Conference on Harmonisation (ICH) E6 (R1); 62 Federal Register 25691 (1997)

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

SIGNATURE PAGE

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

Principal Investigator:
Robert W. Frenck, Jr., M.D.

Signed: _____ Date: _____

Name

Title

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LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
ALS	Antibody Lymphocyte Supernatant
ASC	Antibody-Secreting Cells
BGS	Buffered Glycerol Saline
BMGF	Bill and Melinda Gates Foundation
CCHMC	Cincinnati Children's Hospital Medical Center
CFR	Code of Federal Regulations
CRF	Case Report Form
cfu	Colony Forming Units
CRADA	Cooperative Research and Development Agreement
CRO	Contract Research Organization
DoD	Department of Defense
eCRF	Electronic Case Report Form
ELISPOT	Enzyme-Linked ImmunoSpot
FDA	Food and Drug Administration
FIH	First-in-human
GCP	Good Clinical Practice
HEA	Hektoen Enteric Agar
HLA	Human Leukocyte Antigen
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
ICS	Inventory Control System
ID	Identification
IEC	Independent or Institutional Ethics Committee
IND	Investigational New Drug Application
IRB	Institutional Review Board
LPS	Lipopolysaccharide
MOP	Manual of Procedures
USAMRMC	US Army Medical Research and Materiel Command
n	Number (typically refers to subjects)
PBF	Pilot Bioproduction Facility
PCB	Production Cell Bank
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PI-IBS	Post-Infectious Irritable Bowel Syndrome
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event/Serious Adverse Experience
SMC	Safety Monitoring Committee

LIST OF ABBREVIATIONS

SOP	Standard Operating Procedure
SSP	Study Specific Procedure
SWI	Sterile Water for Injection
US	United States
WRAIR	Walter Reed Army Institute for Research

PROTOCOL SUMMARY

Title:	Dose-Finding Study of Lyophilized *Shigella sonnei* 53G (Lot 1794) Challenge Strain
Abbreviated Title:	*Shigella sonnei* 53G Challenge
Phase:	Phase 1; First-in-human (FIH) study.
Population:	Inpatient study of approximately 60 healthy adults (aged 18 to 49, inclusive at time of participation) who live in the recruiting range of the Cincinnati Children's Hospital Medical Center (CCHMC)**.**
Number of Sites:	**1** Cincinnati Children's Hospital Medical Center (CCHMC), Cincinnati, Ohio **Clinical:** CCHMC **Laboratory:** CCHMC and WRAIR
IND Sponsor:	PATH
Study Sponsor/Funding Mechanism:	Department of Defense (DoD) and PATH
Study Duration:	1-1/2 years
Subject Participation Duration:	6-8 months Screening: 1 to 45 days Inpatient participation: 8 days Follow-up: 180 days (approximately three 1-day follow-up visits and one phone call)
Test Article and Dosing:	The challenge product will be lyophilized *Shigella sonnei* 53G strain: The starting dose is 500 cfu (Doses may be increased or lowered based on results) After a 90 minute fast, subjects will drink 120 ml of sodium bicarbonate just prior to ingesting 30 ml of sterile normal saline 0.9% containing the Shigella inoculums.

Study Objectives:

Primary Objective:

1. Establish a human challenge model of *S. sonnei* 53G infection using a lyophilized formulation of the challenge strain.
2. Identify a dose of lyophilized *S. sonnei* 53G that induces the primary outcome in approximately 60% of subjects with no adverse safety concerns.

Secondary Objective:

1. Estimate quantitative shedding and basic immunogenicity of the challenge strain.
2. Collect and archive blood and fecal samples for systems biology, microbiome, and other omics-based work to be conducted under a separate research protocol in future studies.

Description of Study Design:

Healthy subjects 18-49 years of age (up to 50th birthday) will be admitted to an inpatient unit at CCHMC. Subjects will remain in the inpatient unit for approximately 8 days post-challenge to allow close clinical observation as well as collection of blood, stool, and saliva (cohorts 2-5 only) samples at multiple time points during the inpatient period. On day 5 post-challenge; subjects will be administered ciprofloxacin to eradicate the Shigella from the stool of the subject. Subjects will return to the outpatient clinic on 2-3 occasions post-challenge for clinical check and additional lab collection. The trial will consist of five cohorts; four dose ranging, and one confirmatory cohort. Ten subjects will be enrolled in each dose ranging cohort while up to 20 subjects will be enrolled in the confirmatory cohort.

An adaptive design will be used to determine the dose of *Shigella sonnei* 53G that induces the primary outcome in approximately 60% of subjects. In the first cohort, about 10 subjects will be administered 500 cfu (range 400-600) of *S. sonnei* 53G. Depending on the percentage of subjects who met clinical endpoints, defined as the attack rate (AR), the dose for the second cohort will be adjusted. If the AR is less than 60%, the next cohort (cohort 2) will receive a higher inoculum, not to exceed 1000 cfu, based on deliberations between the investigative team, the research monitor and the Safety Monitoring Committee (SMC). ARs at or above 60%

may result in verification of the attack rate at the same inoculum dose or a decrease in the inoculum to as low as 100 cfu based on deliberations between the investigative team, the research monitor and the SMC. Similar to the second cohort, the dosing for the third and fourth cohorts will be based on the AR in the previous cohort. The results from the four dose ranging cohorts will be reviewed to select the dose to be administered to the confirmatory cohort of up to 20 subjects.

If the targeted AR is not achieved (either too high an AR at the low inoculum or too low an AR at the high inoculum), the results will be evaluated to determine if the dose should be increased or decreased. An iterative process will be used to select the optimal dose with each step reviewed and approved by the safety monitoring committee. An interim safety report will compile findings from the preceding dose group with the PI's interpretation and plan to go forward. The safety monitoring committee must concur with the plan before proceeding. The decision to advance to the next dose level will be based solely on the disease AR and the observed safety profile at the tested dose level.

Estimated Time to Complete Enrollment: Approximately 8 months

Schematic of Study Design

Dose escalation will occur in a stepwise fashion as described in the table below. For the initial dose cohort, enrollment will be limited initially to one subject per day for the first three days. If no unexpected symptoms occur within 24 hours of dosing the third subject, concurrent enrollment of the remaining subjects in the cohort may proceed.

A research monitor, in conjunction with an SMC, established as an advisory committee to the study sponsors, will review the inpatient safety data and any reported SAEs through Day 14 from each dose level prior to making a recommendation for advancement to the next dose level. One of the following recommendations will be made: advancing to the next dose level, halting, additional subjects at the current dose, or decreasing the current dose. The decision will be dependent upon the assessment of safety parameters and by meeting criteria for advancement to the next dose. The safety data to be reviewed by the research monitor and the SMC will include inpatient safety data and all reported serious adverse events (SAEs). Subjects participating in one dose level will not be permitted to participate in a subsequent dose level.

Cohort	**Product**	**Inoculum dose**	**N**
A	*S. sonnei* 53G	500 cfu	10
B	*S. sonnei* 53G	250 cfu or 1000 cfu (based on results of cohort A)	10
C	*S. sonnei* 53G	TBD based on cohort A and B	10
D	*S. sonnei* 53G	TBD based on cohorts A-C	10
E	*S. sonnei* 53G	Based on results of previous cohorts	Up to 20

1. KEY ROLES

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2. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1. Background Information

Diarrheal diseases continue to be major public health problems throughout the world. In 1999, Kotloff et al. conducted a systematic literature review and estimated the global incidence of shigellosis, a major bacterial diarrheal pathogen [1]. This review estimated there to be 164.7 million cases of Shigella per year resulting in approximately 1.1 million shigellosis-related deaths [1]. Shigella is comprised of four species (*S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei)* with *S. sonnei* being the most common species of Shigella-associated diarrhea in developed countries and *S. flexneri* being the most common species of Shigella-associated diarrhea in the developing world [2]. More recent data has estimated mortality rates to be lower; however epidemiological information on incidence rates of Shigella-mediated diarrhea from much of Asia and Africa is missing [3].

Shigella is a low-inoculum infection transmitted by the fecal-oral route with as few as 10 colony forming units (cfu) sufficient to cause disease in highly susceptible people [4]. Due to ease of transmission, multiple secondary cases from an initial case are common [4]. Infections caused by Shigella can range from mild watery diarrhea to severe disease characterized by fever, headaches, abdominal cramps, and dysentery (frequent small volume stools containing blood and mucus). Although Shigella-associated diarrhea typically is self-limiting, infection frequently is debilitating for 5-7 days if untreated [2, 5].

Currently, the mainstays against Shigella are prevention and treatment of infections. Improved sanitation and education on safe eating practices are the principal means of prevention while treatment typically requires administration of antimicrobial therapy [1, 6]. Improving sanitation is difficult, particularly in resource limited areas thus limiting the effectiveness of prevention. Additionally, the emergence of multidrug-resistant Shigella strains has complicated the treatment of shigellosis [7-9]. Thus, alternative methods to prevent infection, such as vaccines, are critically needed.

Humans are the only natural host for Shigella making it more difficult to study the prevention and/or treatment of infections with the organism. While non-human primate models have been developed, inoculums multiple logs higher than needed to cause an infection in humans are needed to reproducibly induce infection. Islam et al. demonstrated that for rhesus monkeys, an intragastric challenge of 2 $x10^9$ cfu of *Shigella dysenteriae* 1 1617 strain was required to induce disease in 4 of 5 monkeys with no previous exposure to Shigella (80% attack rate)[10]. Additionally, of the 5 monkeys who previously received the 2 $x10^9$ cfu dose, only 2 developed symptoms after administration of a 2 $x10^{10}$ cfu dose (60% protection). In comparison, when a 2 $x10^{10}$ cfu dose was administered to monkeys without previous exposure to Shigella; all developed severe dysentery and one monkey died within 24 hours of challenge [10].

Gregory et al. have developed a challenge model of infection for *S. flexneri* in the *Aotus nancymaae* monkey [11]. An oral dose of $1x10^{11}$ cfu of *Shigella flexneri* 2a strain 2457T induced infection in 75% of the monkeys. However, unlike the rhesus model; a previous infection with the challenge organism did not protect against infection when the animals were administered a

second challenge dose 9 weeks after the first [11]. The limitations of the currently available animal models reinforce the need for a safe and reproducible human challenge model.

Two strains of Shigella, *S. flexneri* 2457T and *S. sonnei* 53G, have been most commonly used in human challenge models. As part of a study of a bi-valent Shigella vaccine; subjects from the United States were given 3 doses of vaccine or placebo followed by challenge with 500 cfu of *S. sonnei* 53G 1 month after the third dose of vaccine [12]. Of the 38 healthy control subjects; 20 (53%) developed diarrhea and fever. In a follow-up of the previously described study; 16 placebo recipients were challenged with 500 cfu of *S. sonnei* 53G 1 month after the third dose of placebo. Ten of the 16 subjects (62%) became ill [13]. A third study from the same group compiled results from previous studies to determine if there were a correlation between peak temperature of the subject and severity of the infection [14]. A total of 85 control subjects received 500 cfu of *S. sonnei* 53G and 40 (48%) developed fever and diarrhea [14]. The investigators noted that fever did correlate with more severe illness. However, the duration of symptoms was shorter in the group that developed the higher fevers. In a trial evaluating the cytokine response to Shigella infection; 11 subjects were administered 500 cfu of *S. sonnei* 53G and 6 (55%) became ill [15]. The summary of the above studies, all conducted in the United States, is that approximately 50% of subjects develop diarrhea and/or dysentery (range 40-60%) after ingesting 500 cfu of *S. sonnei* 53G.

A critical step to increase the likelihood of inducing an infection with an enteric organism is neutralization of stomach acid. All of the above studies used skim milk to neutralize stomach acidity. A finding of all the above studies was that the rate of infection plateaued at about 60% of subjects regardless of the dose of the challenge strain administered. In an attempt to increase the attack rate; milk was substituted with sodium bicarbonate [16]. When subjects were administered 10^3 cfu of *S. flexneri* 2457T mixed with sodium bicarbonate; 10 of 14 subjects (71%) developed fever and 9 of 14 (64%) developed diarrhea and/or dysentery [16]. In a second study, subjects were administered 1.4×10^3 cfu of *S. flexneri* 2457T mixed with sodium bicarbonate [17]. Of the 12 subjects, 10 (83%) developed fever along with diarrhea and/or dysentery. Lowering the dose to 1.4×10^2 cfu of *S. flexneri* 2457T lowered the infection rate to 43% (3/7 subjects). A third study confirmed the results of the previous work when 8 of 10 (80%) subjects administered 1.5×10^3 cfu of *S. flexneri* 2457T mixed with sodium bicarbonate developed diarrhea [18]. These studies thus demonstrated that substituting skim milk with sodium bicarbonate could markedly increase the rate of infection caused by *S. flexneri* 2457T. Additionally, the studies suggested a steep dose response curve between administration of 140 and 1400 cfu of the challenge strain.

A recent study evaluated the infectivity of *S. sonnei* 53G when administered to people in Thailand [19]. In ascending cohorts of 12; subjects were administered either 100, 400 or 1600 cfu of *S. sonnei* 53G after drinking 150 mL of a sodium bicarbonate solution. The two lower doses resulted in 6 (50%) subjects in each cohort meeting clinical endpoints while 9 (75%) subjects receiving 1600 cfu met clinical endpoints [19]. These data closely mirror the results of the studies discussed above when subjects were challenged with *S. flexneri* 2457T mixed with sodium bicarbonate and again suggest a rather steep dose response curve for infection between 500 and 1500 cfu as a challenge dose.

While the human challenge studies of Shigella have produced important data, many concerns have been raised about these data including; lack of standard inoculum; lack of reproducibility; small sample size of study subjects; varying outcome definitions and minimal understanding of the immune response to infection [20]. Additionally, the method of preparation of the Shigella inoculum from a bacterial suspension with colony count estimated from a fixed optical density has been cited as a barrier to standardization and reproducibility [20]. The current proposal will address these deficiencies and establish a well characterized model of infection with *S. sonnei* 53G.

2.2 Scientific Innovation

The project will employ many innovative approaches and technology in the development of the challenge model. The first is the use of a lyophilized strain of *S. sonnei* 53G. In previous challenge studies; doses of 53G typically have been grown from thawed glycerol stock or starter culture from one or more earlier passages [17-19]. The confluent bacterial lawns on agar plates are then scraped into some diluent and the dense suspension is adjusted over several iterations to the desired concentration of bacteria. This ad hoc practice is vulnerable to many sources of potential variation, including; the use of different reagent lots between preparations; different bacterial starter cultures; different research personnel, and pipetting and instrument error when handling viscous and; incompletely homogenous suspensions.

In contrast to growing the bacteria from a stock or starter culture; the lyophilized preparation of 53G, grown under GMP conditions, produces a reliable, consistent concentration of bacteria which are fresh and with a low percentage of Form II colonies (See Table 2). *S. sonnei* vaccine candidates have been successfully produced and lyophilized by our team and demonstrated highly reproducible colony counts in a recently completed series of *S. sonnei* vaccine trials at Cincinnati Children's Hospital Medical Center (CCHMC) (unpublished data). The successful evaluation of the *S. sonnei* 53G product will validate the importance of having the lyophilized strain and possibly encourage the manufacture of a lyophilized *S. flexneri* 2a challenge product. The availability of standardized cGMP challenge strains of both *S. sonnei* and *S. flexneri*, the 2 most common circulating Shigella serotypes, will accelerate the development of a bivalent or trivalent Shigella vaccine that has the potential to control 70-80 percent of shigellosis worldwide.

An adaptive design will be used in this study. Rather than a priori determining the dose in all study groups; subsequent doses are based on the response to previous doses. This approach has been shown to more rapidly identify the dose of interest which results in minimizing exposure of subjects to the risks of the study as well as maximizing utilization of time and resources. We have successfully used such a design in a study to determine the dose of norovirus required to induce infection in at least 50% of subjects administered the virus (unpublished data). By using the adaptive design, we also will maximize the number of subjects receiving the dose of interest which will improve the power of the study.

Previous human challenge studies have demonstrated that some individuals (immunologically naïve) are resistant to enteric infection. One possible explanation is the microbiome of the host. The human gut is composed of trillions of symbiotic bacteria which contribute to the normal host physiology including host metabolic signaling and immune pathways. Alterations in the gut flora

in response to enteric pathogens have also been noted; but the complexity of the microbiome and its role in both protection against and response to infection remains to be characterized. A better understanding of the changes in the microbiome associated with Shigella infections and treatment of the infection may allow us to elucidate the biological mechanisms that underlie pathogenesis and point to potential opportunities for prevention and treatment of Shigella.

Another critical area to explore is the "systems biology" to an infection with Shigella. In contrast to previous "reductionist" methods of study; systems biology is a field of study that focuses on complex interactions within biological systems, using a holistic approach. Studying the "signature of infections"; it may be possible to predict people immune to a specific microorganism as well as who may be at increased risk for developing severe infection. Also, this approach may allow us to better understand the immune response to natural infections which could result in vaccines targeted to stimulate those portions of the immune system. We have discussed the above proposal with Dr. Bali Pulendran of Emory, and member of the Systems Biology/Systems Immunology Consortium of the Vaccine Accelerator Platform.

2.3 Potential Risks and Benefits

2.3.1 Potential Risks

The principal risk is also the primary objective of the study; shigellosis. Illness caused by Shigella ranges from mild (watery diarrhea being the main symptom) to severe (fever and abdominal cramping with frequent small volume blood streaked stools containing mucus). Complaints may include; nausea, vomiting, headache, abdominal gurgling or gas, anorexia, muscle and/or joint aches and malaise. Symptoms typically are self-resolving in 5-7 days. However, administration of an effective antibiotic will significantly reduce, or eliminate, symptoms within 24 hours of initiation. Unlike infections with Enterotoxigenic *E. coli* (ETEC), a common cause of Travelers Diarrhea; Shigella is not commonly associated with significant dehydration as stool volume typically is not large. The *S. sonnei* 53G challenge strain is known to be susceptible to multiple antimicrobial agents including TMP-SMX and quinolones. There is a risk of bacteremia, although Shigella bacteremia rarely occurs in adult populations with wild-type Shigella [2].

Reiter's syndrome or post-dysenteric reactive arthritis, with ocular and/or urethral inflammation, which has an onset typically 1 to 3 weeks after the onset of diarrhea, has been reported to occur in approximately 2 to 3 percent of Caucasian patients with shigellosis, where the risk of arthritis was proportional to the severity of the disease [21]. Some data suggest that the risk of reactive arthritis following shigellosis may be higher in persons carrying the Human Leukocyte Antigen (HLA)-B27 antigen [22]. Therefore, subjects who were HLA-B27 positive will be excluded from participation.

Recent studies suggest an increased risk of post-infectious irritable bowel syndrome (PI-IBS) following bacterial enteritis, with limited studies associating Shigella infection specifically with these sequelae [23, 24]. PI-IBS, a functional bowel disorder characterized by unexplained abdominal discomfort or pain associated with changes in normal bowel patterns, has been described in a recent systematic review to occur 6-7 times more frequently after an acute

enteric infection compared to similar matched controls without such a history [23]. To minimize study risks, subjects with a history of abnormal bowel patterns who might be at higher risk for these post-infectious sequelae will be excluded. Pre-defined criteria to ensure early treatment as appropriate also may reduce further the risk of post-infectious sequelae as has been observed with reactive arthritis [22] and possibly would reduce the risk associated with PI-IBS given the positive association between diarrheal illness duration and PI-IBS risk. Mild gastrointestinal symptoms may occur due to ingestion of the bicarbonate solution.

To avoid potential risks; pregnant or nursing women will be excluded from the study and women of childbearing potential will be counseled against becoming pregnant during their participation in the study.

Potential risk from Ciprofloxacin and Bactrim (sulfamethoxazole and trimethoprim):

Ciprofloxacin, a broad spectrum antibacterial agent belonging to the fluoroquinolone family of antibiotics, is the first-line treatment agent for all subjects on day 5 of the clinical trial. Ciprofloxacin is generally well-tolerated. The most frequently reported drug related events for all indications of ciprofloxacin therapy have been nausea, diarrhea, abnormal liver function tests, vomiting, and rash. Most of the AEs reported were described as only mild or moderate in severity, abated soon after the drug was discontinued, and required no treatment. Fluoroquinolones, including ciprofloxacin, are associated with an increased risk of tendonitis and tendon rupture in all ages. Other symptoms rarely seen include hypersensitivity, dizziness, pseudomembranous colitis and peripheral neuropathy.

The most common AEs of Bactrim (alternate treatment agent) are gastrointestinal disturbances (nausea, vomiting, and anorexia) and allergic skin reactions (such as rash and urticaria).

Other risks:

There is the risk of pain, hematoma, or infection at the site of venipuncture. There is also a risk of the study staff acquiring the infection from subjects who were administered the *S. sonnei* 53G. Therefore, proper personal protective equipment will be worn by staff, and good personal hygiene such as frequent hand washing will be followed by staff and subjects.

2.3.2 Known Potential Benefits

Study subjects will have no direct benefit from study participation. However, a successful outcome of the trial could lead to the development of a model that will allow easier and more reproducible evaluation of preventive and therapeutic measures against disease due to *S. sonnei*.

3. OBJECTIVES

3.1 Primary Objective

1. Establish a human challenge model of *S. sonnei* 53G infection using a lyophilized formulation of the challenge strain.
2. Identify a dose of lyophilized *S. sonnei* 53G that induces the primary outcome in approximately 60% of subjects with no adverse safety concerns.

3.2 Secondary Objectives

1. Estimate quantitative shedding and basic immunogenicity of the challenge strain.
2. Collect and archive blood and fecal samples for systems biology, microbiome, and other omics-based work to be conducted under a separate research protocol in future studies.

4. STUDY DESIGN

An adaptive design will be used to determine the dose of *Shigella sonnei* 53G that induces the primary outcome in approximately 60% of subjects.

An unexpected shock-like illness of unknown etiology occurred in one of six subjects at Johns Hopkins Center for Immunization Research at 10 hours after inoculation with *Shigella sonnei* strain 53G prepared from frozen stock. Therefore, for the initial dose cohort, enrollment will be limited initially to one subject per day for the first three days. If no unexpected symptoms occur within 24 hours of dosing the third subject, concurrent enrollment of the remaining subjects in the cohort may proceed.

In the first cohort, about 10 subjects (minimum 8) will be administered 500 cfu (range 400-600) of *S. sonnei* 53G. Depending on the attack rate (AR) of the starting inoculum (defined as the percentage of subjects who met clinical endpoints); the dose for the second cohort will be adjusted. If the AR is less than 60%, the next cohort (cohort 2) will receive a higher inoculum, not to exceed 1000 cfu, based on deliberations between the investigative team, the research monitor and the SMC. ARs at or above 60% may result in verification of the attack rate at the same inoculum dose or a decrease in the inoculum to as low as 100 cfu based on deliberations between the investigative team, the research monitor and the SMC. Using the process described above; the dosing for the third and fourth cohorts will be based on the AR in the previous cohort. The results from the four dose ranging cohorts will be reviewed to select the dose to be administered to the confirmatory cohort of up to 20 subjects.

If the targeted AR is not achieved (either too high an AR at the low inoculum or too low an AR at the high inoculum); the results will be evaluated to determine if the dose should be increased or

decreased. An iterative process will be used to select the optimal strain and dose with each step reviewed and approved by the SMC.

An interim safety report will compile findings from the preceding dose group with the PI's interpretation and plan to go forward. The research monitor, in collaboration with the SMC, must concur with the plan before proceeding. The decision to advance to the next dose level will be based solely on the disease attack rate and the observed safety profile at the tested dose level.

Screening will be done on days -45 to -2, with admission to the inpatient unit on day -1. Challenge will occur on day 0 and discharge is planned on day 8 but the exact date will be when the subject meets discharge criteria. Two to three additional subjects per cohort will be screened and deemed "alternates". The "alternates" will be admitted to the inpatient unit along with the "primary" cohort. Prior to dosing, the eligibility of subjects in the "primary" cohort will be re-confirmed. In the event a subject in the "primary" cohort is unavailable, or becomes ineligible; that subject will be replaced by an alternate. Any alternate not needed to become a member of the "primary" cohort will be discharged from the unit prior to administration of the challenge dose. For subjects receiving a challenge dose, following discharge from the inpatient unit, there will be clinic visits on days 14±2, 28±2, and 56±4, and a safety follow-up call will occur at day 180±14 after challenge.

During the inpatient stay and post challenge administration, subjects will be evaluated at least daily to assess for any symptoms or adverse events. Additional assessments will include at the minimum: daily measurement of vital signs (temperature, pulse, and blood pressure); daily history directed physical examinations; the collection and grading (for consistency) of each stool; and weighing of all loose or watery stools. Stool specimens or rectal swabs (if unable to produce a stool) will also be evaluated for grossly visible blood and tested for occult blood using hemoccult. Hemoccult testing may be limited to two samples per day if positive. Additionally, one sample per day will be cultured for the presence of the challenge strain. All subjects will receive antibiotic therapy 5 days after challenge inoculation (unless meeting criteria for early treatment).

Subjects will be eligible for discharge from the inpatient unit on day 8 if they have started their antibiotic treatment on day 5 and passed 2 consecutive stools culture-negative for *S. sonnei* at least six hours apart. In the event stool cultures are still growing *S. sonnei*; subjects will remain in the inpatient unit until their stool is culture-negative.

Following an evaluation and history directed physical examination; subjects will be discharged, with follow-up on study days 14±2 28±2 and 56±4 to provide additional stool, saliva (cohorts 2-5 only) and blood specimens, according to the Schedule of Events in Appendix A, for safety checks or immunology monitoring. Subjects will be given an ice-pack and cooler for collection of a stool sample at home and instructed to bring the sample with them to the follow-up visit. At approximately study day 180, the subjects will be contacted by phone for supplemental surveillance to track the occurrence of any medically significant new chronic illnesses, pregnancy, or serious health event.

Any subject who wishes to withdraw early from the inpatient portion of the study will be treated with antimicrobials and should have two negative stool cultures before they are discharged. These subjects will be requested to adhere to the same follow-up schedule as all other subjects.

Dose escalation will occur in a stepwise fashion and will be dependent upon the assessment of safety parameters and by meeting criteria for advancement to the next dose. A Safety Monitoring Committee (SMC) established as an advisory committee to the study sponsors for this study, along with the research monitor, will review inpatient safety data and SAEs through day 14 from each dose level prior to making a recommendation for advancement to the next dose level. The SMC would recommend one of the following alternatives: advancing to the next dose level, halting, additional subjects at the current dose, or decreasing the current dose. The next inpatient group will not be admitted until a minimum of 4 weeks have elapsed from the time of the preceding group's challenge to ensure adequate time for safety monitoring. Subjects participating in one dose level will not be permitted to participate in a subsequent dose level.

The inpatient research unit where this study will be conducted is part of CCHMC and is well equipped and suited for clinical research studies on adult subjects. Only clinical research studies are performed on this unit which is capable of accommodating up to 24 inpatients at a time. Rooms can be utilized as private or semi-private rooms (depending on the needs of particular studies). Each room has its own private bathroom. The inpatient unit has controlled access. Our research clinic, located on the same floor as the inpatient unit, will be used for the outpatient portion of this study.

To minimize bias on study outcomes, an independent outcome adjudication committee, comprised of up to 3 individuals, independent of the study sponsors and investigative team, will review all study endpoints (diarrhea, fever, etc.) after study completion (defined as after day 180 of the final cohort). The role of the committee will be to (1) review and confirm all primary endpoint cases; (2) review all protocol-specified entry criteria, adherence, and compliance issues to ascertain classification in the per-protocol and other study populations; and (3) provide guidance regarding secondary and other endpoint classifications to include agreement on objective criteria for classification of endpoints. Specific duties and responsibilities will be outlined by charter prior to the start of the study. The members of the adjudication committee may also serve as members of the SMC.

4.1 Study Outcome Measures

4.1.1 Primary Outcome Measures

Shigellosis is defined as shedding of *S. sonnei* in the stool accompanied by moderate-severe diarrhea and/or dysentery along with moderate fever or one or more severe intestinal symptoms. All stools will be collected, weighed and graded to assess for diarrhea. Only Grade 3, 4 and 5 stools will be considered in the criteria for diarrhea. Grade 3, 4 and 5 stools within any 24 hour period will be added to determine the highest severity grade for the episode. When there are no loose stools for 48 hours, the episode will be considered resolved. The end date

for the episode will be the date and time of the last Grade 3, 4, or 5 stool. The clinical disease endpoint is shigellosis and the primary clinical endpoints are shown below:

- **Diarrhea**-
  - **Moderate**- 4-5 loose or watery Grade 3-5 stools or 400-800 gram/Grade 3-5 stools per 24 hours
  - **Severe**- 6 or more loose or watery Grade 3-5 stools or >800 gram/Grade 3-5 stools per 24 hours or requires medical intervention
- **Dysentery**- a Grade 3, 4, or 5 stool with gross blood on at least 2 occasions and reportable constitutional symptoms.
- **Moderate Fever**- Oral temperature of ≥38.5°C
- **Symptoms** - one or more severe intestinal symptoms

 Occurrence of abnormal clinical laboratory values within 11 days post challenge.

The severity of these outcomes will be graded based on the definitions outlined in the AE Section 9.2.1.

4.1.2 Secondary Outcome Measures

1. Immunogenicity
   a. Systemic Immunogenicity:
      i. Serum IgA, IgM and IgG response to Shigella antigens: *S. sonnei* LPS, *S. sonnei* Invaplex, IpaB, IpaC and IpaD
   b. Mucosal immunogenicity:
      i. IgA and IgG Antibody Secreting Cells (ASC) and Antibody in Lymphocyte Supernatant (ALS) response to Shigella antigens: *S. sonnei* LPS, *S. sonnei* Invaplex, IpaB, IpaC, IpaD; Fecal IgA response to Shigella antigens: *S. sonnei* LPS, *S. sonnei* Invaplex, IpaB, IpaC and IpaD; and Salivary IgA response to Shigella antigens.
   c. Cell Mediated Immunogenicity: Lymphocyte proliferation after *ex vivo stimulation* of PBMCs with Shigella-related antigens (IpaB, IpaC, IpaD, Invaplex, LPS, and appropriate controls). The phenotype of the immune response will be characterized as Th1 and Th2 based on the cytokines secreted during the proliferation assay using a multiplex assay format. Cytokines to be measured include, but not limited to; IL-2, IL-4, IL-5, IL-10, IL-12, IL-17, IL-18 and IFN-γ for Th1/Th2. These investigational assays are required to better understand the immune response to Shigella infections.
   d. B Memory Cell assays: B memory cells (B_M) are long lived plasma cells that provide an anamnestic or recall response in the host upon reexposure to the same antigen. The response is usually of greater magnitude, is faster and

better quality and has been correlative of protection in live Shigella vaccine studies [25, 26]. In this study IgG and IgA B_M cell responses will be measured as Enzyme-Linked ImmunoSpot (ELISPOT) assays with PBMCs to *S. sonnei* LPS, *S. sonnei* Invaplex, IpaB, IpaC and IpaD. PBMCs will be expanded *in vitro* with appropriate mitogens prior to measuring B_M responses to specific Shigella antigens.

e. Expanded Immunology and Systems Biology: Specific samples will be collected as part of this study to support future evaluations in systems biology. Cells and serum samples will be collected for use in a variety of omics-based work such as transcriptomic and cytokine analysis.

2. Fecal shedding of Shigella – Daily during the inpatient stay, a sample of stool will be screened for the presence of the *S. sonnei* strain. Refer to the Manual of Procedures (MOP). If there is no stool sample, collect a rectal swab and streak on HEA as described in the MOP.

4.1.3 Exploratory Outcome

4.1.3.1 Stool Polymerase Chain Reaction (PCR) to assess quantitative stool burden of *S. sonnei*

5. STUDY ENROLLMENT AND WITHDRAWAL

5.1 Subject Inclusion Criteria

To be eligible to participate, each subject must fulfill all of the following criteria:

1. Male or non-pregnant female between 18 and 49 years of age (inclusive).
2. General good health defined as (a) no significant medical illness, (b) no clinically significant physical examination findings and (c) no screening laboratory values significantly outside the normal limits of the testing laboratory within 45 days of challenge.
3. Demonstrate comprehension of the protocol procedures and knowledge of study by passing a written examination (pass grade ≥70%) on day -1.
4. Willing to sign an informed consent form (ICF).
5. Willingness to participate for an inpatient stay lasting up to 11 days and an outpatient follow-up lasting 6 months from challenge.
6. Willing to not smoke during the inpatient stay.
7. Available for all planned follow-up visits.
8. Negative serum pregnancy test at screening and negative urine pregnancy test on the day of admission to the inpatient phase for female subjects of childbearing potential.

Females 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) within two months before challenge through day 180. Abstinence is acceptable. A woman is eligible if she is monogamous with a vasectomized partner.

9. Willing to not donate blood for up to 6 months after completion of the inpatient phase of the study.
10. Willing to refrain from participation in another investigational vaccine or drug trial at least until after completion of the 6 month follow-up safety call.

5.2 Subject Exclusion Criteria

Subjects who meet any of the following criteria at baseline will be excluded from participation:

1. Presence of a significant medical condition (e.g. psychiatric conditions, alcohol or illicit drug abuse/dependency, or gastrointestinal disease, such as peptic ulcer, symptoms or evidence of active gastritis or gastroesophageal reflux disease, inflammatory bowel disease), or other laboratory abnormalities which in the opinion of the investigator precludes participation in the study.
2. Immunosuppressive illness or IgA deficiency
3. Positive serology results for HIV, HBsAg, HCV, or RPR (syphilis) antibodies.
4. Evidence of inflammatory arthritis on exam and/or HLA-B27 positive.
5. Family history of inflammatory arthritis.
6. Significant abnormalities in screening lab hematology or serum chemistry, as determined by PI.
7. Allergy to fluoroquinolones or trimethoprim-sulfamethoxazole
8. Fewer than 3 stools per week or more than 3 stools per day as the usual frequency.
9. History of diarrhea in the 2 weeks prior to planned inpatient phase
10. Use of antibiotics during the 7 days before receiving the challenge inoculum dosing
11. Use of prescription and/or OTC medications that contain Imodium, acetaminophen, aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs, during the 48 hours prior to investigational product administration
12. Travel within two years prior to dosing to countries where Shigella infection is endemic.
13. Use of any medication known to affect the immune function [e.g., oral steroids, parenteral steroids, or high-dose inhaled steroids (>800 µg/day of beclomethasone dipropionate or equivalent and others): nasal and topical steroids are allowed] within 30 days preceding receipt of the challenge inoculum or planned use during the active study period.

14. Serologic evidence of *Shigella sonnei* (titer > 1:2500)
15. A positive urine test for opiates
16. A chronic disease (such as hypertension, hyperlipidemia or anxiety/depression) for which doses of prescription medications are not stable for at least the past 3 months.
17. Have immunocompromised household contacts.
18. A clinically significant abnormality on physical examination, including a systolic blood pressure >140 mm Hg or diastolic blood pressure >90 mm Hg, or a resting pulse >100 beats/min or <55 beats/min (<50 beats/min for conditioned athletes).
19. Pregnant, nursing, or plan to become pregnant within 6 months of receipt of the study product.
20. In the 4 weeks following challenge, subject will be living with or having daily contact with elderly persons aged 70 years or more, diapered individuals, persons with disabilities, children <2 years old, or a woman known to be pregnant or nursing, or anyone with diminished immunity. This includes contact at home, school, day-care, nursing homes, or similar places.
21. Work in a health care setting, day care center, or as a food handler in the 4 weeks following the challenge with *S. sonnei*.
22. Use of any investigational drug or any investigational vaccine within 60 days preceding challenge, or planned use during the 6 months after receipt of the study agent.
23. Have received a licensed, live vaccine within 28 days or a licensed inactivated vaccine within 14 days of receiving the challenge inoculum.
24. Inability to comply with inpatient rules and regulations.
25. Has any other condition that, in the opinion of the Investigator, would jeopardize the safety or rights of a participant or would render the subject unable to comply with the protocol.
26. Received blood or blood products within the past six months.

6. TREATMENT ASSIGNMENT PROCEDURES

6.1 Withdrawal

6.1.1 Reasons for Withdrawal

A subject may be withdrawn from study participation if any clinical AE, laboratory abnormality, medical condition or situation occurs such that continued participation in the study would not be in the best interest of the subject. The US Food and Drug Administration (FDA) also may elect to discontinue the study for safety reasons. The study may also be terminated at the direction of the study sponsors.

Subjects are free to withdraw from participation in this study at any time upon request, for any reason, specified or unspecified and without penalty or loss of medical benefits to which the subject is otherwise entitled. If voluntary withdrawal occurs, the subject will be asked to complete a final visit for safety evaluation and will receive appropriate care under medical supervision if symptoms of any AE related to participation in the study are continuing. The subject will be followed until the AE is resolved or until the subject's condition becomes stable.

Subjects may be withdrawn from the study for any of the following reasons:

- Withdrawal of consent, deviation from the protocol, incidental illness, or an AE occurs such that continued participation in the study would not be in the best interest of the subject.
- The reason for withdrawal should be documented in the subjects' records, including the date of withdrawal/final assessment.

6.1.2 Handling of Withdrawals

If a subject decides to leave the inpatient facility after receipt of the challenge strain but before planned discharge; they will be strongly encouraged to at the minimum complete the antibiotic treatment before withdrawing from the study. Subjects who choose to withdraw from the inpatient portion of the study will be encouraged to complete all outpatient visits according to the same follow-up schedule as all other challenged subjects.

6.2 Termination of Study

During the clinical trial, if any subject develops an SAE determined to be related to study participation; the study will be reviewed by the SMC, along with the research monitor to determine whether the study will proceed or be discontinued. The FDA also may elect to discontinue the study for safety reasons. The study may also be terminated at the direction of the study sponsors.

7. INVESTIGATIONAL PRODUCT

7.1 Study Products Description

S. sonnei 53G was initially isolated from a child with diarrhea in Tokyo in 1954. The seed was maintained at the Center for Vaccine Development, University of Maryland. A master cell bank (MCB) (BPR-327-00, Lot 0593) was manufactured by the Pilot Bioproduction Facility (PBF), Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD, U.S. The lyophilized 53G strain of *S. sonnei* was manufactured under current Good Manufacturing Practice (cGMP) conditions at the WRAIR PBF. Testing completed on *S. sonnei* 53G are listed in Table 1.

7.1.1 Acquisition

For *S. sonnei* 53G; MCB seeds and Production Cell Bank (PCB Lot 0599) seeds were available for use and stored at the WRAIR Pilot Bioproduction Facility as 1 mL glycerol cultures. A single vial of PCB was used to carry out a 30-liter fermentation, and the contents were lyophilized after the cultures were suspended in a cryopreservative. The lyophilized vials are stored at -80ºC ± 10ºC at the PBF.

Table 1. Tests Completed on *S. sonnei* 53G

Test	Result
Final Container Lot Evaluation	Pass; White cake with no visible particulates
Viability and stability	Pass; > 4×10^8 cfu/vial and > 50% Form I cfu (WRAIR Pilot Bioproduction Facility and WRAIR Dept. of Live Shigella Vaccines)
Biochemical identification	Pass; *Shigella sonnei* (WRAIR Multidrug-resistant organism Repository and Surveillance Network)
Antibiotic Sensitivity	Pass; Sensitive to expected antibiotics, including ciprofloxacin, TMP-SMX, and tetracycline (WRAIR Multidrug-resistant organism Repository and Surveillance Network)
pH	Report as tested; pH 6.5 (WRAIR Pilot Bioproduction Facility)
Moisture Content	Pass; < 3% (BioReliance)
Invasion assay	Pass; Invasive in HeLa cells (WRAIR Dept. of Live Shigella Vaccines)
Plaque assay	Pass; Plaque-positive (WRAIR Dept. of Live Shigella Vaccines)
Virulence assay	Pass; Virulent, Sereny-positive in the guinea pig keratoconjunctivitis model (WRAIR Dept of Live Shigella Vaccines)
Presence of lytic bacteriophage	Pass; Negative (PATH Vaccine Solutions and Charles River Laboratories)
Microbial Limits Test	Pass; Meets USP <61> and <62> criteria of acceptance for the Microbiological Examination of Non-Sterile Products for Microbial Enumeration Test for Yeast and Mold and Tests for Specified Microorganisms: Bile-tolerant Gram-negative bacteria, Escherichia coli, Salmonella spp., Pseudomonas aeruginosa, Staphylococcus aureus, Clostridia spp., and Candida albicans (BioScreen Testing Services)

Product Storage, Stability, and Expiration

Vials containing the challenge strain will be stored in the Investigational Pharmacy in a -80ºC ± 10ºC freezer that is under a temperature-monitoring program. On the day of challenge; lyophilized frozen vials of *Shigella sonnei* 53G strain (Lot 1794) will be prepared by an Investigational Pharmacist.

The viability and stability of *Shigella sonnei* 53G has been measured by removing vials from storage and doing colony counts as well as evaluating the percentage of Form I phenotype by colony immunoblots with monoclonal antibodies to either IpaB or IpaC. These studies were carried out at the PBF and the Department of Live Shigella Vaccines. Viability of Lot 1794 and stability testing is scheduled to occur annually. The data for *Shigella sonnei* 53G are given in **Table 2**.

Table 2. Viability and Stability Testing of *Shigella sonnei* 53G

Date	Viability (cfu/vial)	Stability (% Form I cfu)
2013 Feb 25	**3.9×10^9**	**> 80%**
2013 Jun 3	**3.2×10^9**	**> 80%**
2013 Aug 26	**2.8×10^9**	**> 80%**
2013 Nov 25	**5.2×10^9**	**> 80%**
2014 Feb 26	**1.9×10^9**	**> 80%**
2014 Aug 13	**3.6×10^9**	**> 80%**
2015 Mar 04	**4.8×10^9**	**> 80%**

Microbiological materials

As detailed in the Manual of Procedures (MOP), WRAIR will ship specialized microbiological materials to CCHMC for training and clinical trial use.

7.1.2 Preparation/Handling

Lyophilized, frozen vials of *S. sonnei* 53G will be maintained in Investigational Pharmacy and stored at -80ºC ± 10ºC until the day of challenge. On the day of challenge, the requisite number of vials will be removed from the freezer and placed on ice and allowed to thaw for 30 minutes. Then 2 mL of cold sterile water for injection (SWI) will be added to each vial. To ensure complete rehydration of the challenge strain along with homogeneous mixing of each vial's contents; the vials will remain on ice for another 15 minutes with intermittent swirling of the suspension. All vials then will be combined and diluted in cold, sterile normal saline 0.9% to

arrive at the desired concentration. The diluted challenge material will be kept on ice until administration. At the time of reconstitution, an aliquot will be removed for testing by colony count to be able to document the actual dose administered to the subjects. Plating and colony count determination will be performed as described in the MOP regarding culturing of stool samples for Shigella. Time of reconstitution will be noted. The maximum hold time is 2 hours on ice between the reconstitution and administration of the challenge product to the subjects.

One mL of the challenge solution at the appropriate concentration will be added to 30 mL of sterile normal saline 0.9%. Two grams of sodium bicarbonate (NaHCO3) in 120 mL of SWI will be placed in a second cup.

7.1.3 Administration

Subjects will fast for 90 minutes prior to receiving the challenge inoculum and 90 minutes after challenge. At the indicated time, the subjects will be gathered together for dose distribution. Subjects will drink the 120 mL of sodium bicarbonate to neutralize gastric acidity and then drink the challenge suspension within 5 minutes of drinking the sodium bicarbonate. Care will be taken to ensure that a minimum amount of time, not to exceed 2 hours, is spent between the reconstitution of the challenge inoculum and the oral administration of the inoculum to the subjects.

Shigella sonnei 53G is classified as a BSL-2 category organism. Personnel will take appropriate precautions for working with BSL-2 organisms and wear proper protective equipment such as lab coats, gloves and, if necessary, masks to shield themselves and others from spills and inadvertent mishaps. Spills will be treated according to SOP (Standard Operating Procedure). All unused reconstituted inoculum and materials containing the bacteria will be disposed of properly in biohazard bags. All exposed materials and all disposable materials will be discarded in the biohazard waste for pick up and decontamination as per CCHMC policy.

7.1.4 Accountability/Final Disposition for the Investigational Products:

Study personnel will maintain inventory of *Shigella sonnei* 53G inoculum according to the SOP "Investigational Product Accountability" of the CCHMC Investigational Drug Service.

The FDA requires accounting for the disposition of all investigational products. The investigator is responsible for ensuring that a current record of product disposition is maintained and dispensed only at an official study site by authorized personnel as required by applicable regulations and guidelines. Records of product disposition as required by federal law consist of the date received, date administered, quantity administered, and the subject to whom the drug was administered.

The pharmacist will be responsible for maintaining accurate records of the shipment and dispensing of the study product, and all efforts will be made to protect the integrity of the test article. The pharmacy records must be available for inspection by the sponsor's representative and is subject to inspection by a regulatory agency (e.g., FDA) at any time.

At the termination of the study, all unused product must be returned to the study sponsor or designee (or destroyed if not feasible to return). A written explanation will be required for any product not returned to the study sponsor, stating the reason it was not returned. Study vials and other contaminated materials will be disposed of using appropriate biohazard precautions. Any test article that pharmacy personnel are instructed to dispose of will be destroyed per site destruction policies.

7.1.5 Ancillary Supplies Description

Oral administration of an antibiotic will be started for all subjects on study day 5 unless they meet the requirements for early treatment (see Section 8.1.4). This treatment is to ensure clearance of the challenge organism from their bodies prior to discharge. The first line treatment is ciprofloxacin 500 mg BID x 3 days; the alternate treatment is trimethoprim-sulfamethoxazole (Bactrim) 160/800 mg BID x 5 days. Ciprofloxacin is a broad-spectrum antibacterial agent belonging to the fluoroquinolone family of antibiotics. Bactrim is a sulfonamide antibiotic combination of trimethoprim and sulfamethoxazole used in the treatment of a variety of bacterial infections. Possible AEs to ciprofloxacin and trimethoprim-sulfamethoxazole are described in Section 2.3.1.

8. STUDY SCHEDULE

A Study Schedule of Events is provided in Appendix A.

8.1 Clinical

8.1.1 Screening (day -45 to -2) (may occur in 2-3 visits)

- Presentation of complete description of study to subjects
- Review inclusion/exclusion criteria
- Sign ICF
- Medical interview
- Medical history
- History of all current medications as well as those taken within the past 28 days
- Vital signs (temperature, pulse, and blood pressure) will be taken and recorded
- Complete physical examination
- Collect lab samples as described in Section 8.2.1

Subjects, who meet all eligibility criteria and sign the study consent form, will be eligible for enrollment in the study. A low creatinine value, a low ALT value and/or a low AST value are/is acceptable for study inclusion. No lower limits of lab values for creatinine, ALT, and AST are

considered to be clinically significant. Acceptable laboratory values are set forth in Appendix B. Subjects will be informed of their screening test results by phone. Those with results that exclude them from eligibility will be given the test results by phone unless the results are sensitive in nature. Subjects who have a positive HIV, Hep B or C test will be asked to return to the clinic to receive their results. Excluded subjects will be advised to seek further evaluation from their primary care provider. Subjects that are found positive for HLA-B27 will be counseled on their risk for ankylosing spondylitis or related diseases and referred to the primary care provider for further questions.

To ensure comprehension of the study, all subjects will have to pass a written examination before they are eligible for inclusion (minimum passing grade is 70%). Participants will be given a sample test at the time of screening. On the day of admission to the inpatient unit, a written test will be administered which the participants will have 2 chances to pass successfully. If subjects are unable to score 70% or greater on the test after the second attempt, they will be excluded from the study.

8.1.2 Admission day (study day –1)

After admission and prior to receipt of the challenge inoculum:

- A complete physical examination will be performed by a study investigator
- Inclusion and exclusion criteria will be reviewed to reassess ongoing eligibility
- Comprehension test will be administered
- Medical interview
- Medical history will be reviewed
- Concomitant medications will be reviewed
- Vital signs (temperature, pulse and blood pressure) will be taken and recorded
- A urine pregnancy test (βHCG) will be performed for all female subjects
- Stool will be collected and sent to the lab as described in Appendix A and Section 8.2.2.6 for baseline stool sample for fecal IgA. Challenge is not dependent on results but efforts will be made to collect 2 samples = between day -5 and day 0 prior to challenge
- Blood will be collected for PBMC, serology, cytokines and systems biology
- Saliva will be collected (cohorts 2-5 only) for salivary IgA between day -1 and day 0 any time prior to challenge

8.1.3 Challenge day (study day 0)

Challenge administration

Study investigator will conduct a medical interview and physical examination and a review of inclusion/exclusion criteria, concomitant medications, and medical history. Subjects will fast for 90 minutes prior to receiving the challenge inoculum and 90 minutes after receipt. Prior to administration of the challenge product; subjects will drink approximately 120 mL solution of bicarbonate buffer to neutralize the stomach acidity. Within 5 minutes, subjects will drink approximately 30 mL of the *S. sonnei* suspension. Challenge preparation is described in Section 7.1.2. If any subject vomits following ingestion of the study agent, they will not be re-dosed. A note will be made in the study record regarding the apparent amount of the vomitus (small, moderate or large). The subject still will continue in the study and undergo all study related procedures.

Post Challenge

Subjects will be closely monitored for any symptoms/events occurring within the first 90 minutes post challenge. The following will be conducted and recorded after 90 minutes:

- Physical assessment: Vital signs (temperature, pulse, and blood pressure) will be checked and history targeted physical exam will be conducted.
- Adverse Events: Symptoms will be assessed for adverse events and concomitant medications will be reviewed.

8.1.4 Inpatient stay (study days 0-8).

Daily clinical assessment: Each study day will be considered midnight to 23:59. At least daily, a study investigator will assess every subject and administer a targeted questionnaire to capture symptoms/signs of illness (including diarrhea, abdominal pain/abdominal cramps, gas, anorexia, nausea, headache, myalgia, malaise, arthralgia, fever, vomiting). Additionally, based on symptomatology; a history-directed physical examination will be performed. Study staff will take vital signs, (temperature, pulse, and blood pressure) and review the subject stool log with investigator. Additional medically indicated clinical investigations will be performed based on the study physicians' judgment. Illness labs may be collected on any participant experiencing Grade 3 or higher illness symptoms and may include chemistry (sodium, potassium, BUN, creatinine total bilirubin, ALT, AST) and hematology (WBC, hemoglobin, neutrophil and lymphocyte count, platelets) evaluations. A urine dipstick may be performed to capture urine specific gravity results in real time.

A study physician will be on-call 24 hours/day. Adverse events, medical history and concomitant medications will be reviewed daily and entered into the source documents.

Subjects will be asked to collect all stools and bring them to the study staff. Qualified medical personnel will evaluate the stool for consistency and the presence/absence of visible blood and/or mucus. Stools that are loose or watery (grades 3-5) will be weighed, and if there is visible blood, a hemoccult test will be performed to confirm presence of occult blood. Hemoccult testing may be limited to two samples per day if positive. Study staff will record the information in the subject's source document. If a subject is unable to produce a stool by midnight, a rectal swab will be obtained.

Saliva will be collected at several time points during the inpatient stay, according to the Schedule of Events in Appendix A.

To assess diarrhea grade, study personnel will use the following definitions for grading of stool samples:

- Grade 1 (Normal stool)– Firm, tootsie roll consistency
- Grade 2 – Soft, pudding consistency, not firm but holds some shape
- Grade 3 (Loose stool)– Takes the shape of the container, thick gravy consistency/brown watery, opaque watery, chocolate milk consistency
- Grade 4 (Watery stool)- Opaque colored, watery consistency
- Grade 5- “rice water”, soapy water consistency

Assessment and Management of Diarrhea, Vomiting, Dysentery, and Dehydration

Only Grade 3, 4 and 5 stools will be considered in the criteria for diarrhea. Grade 3, 4 and 5 stools within any 24 hour period will be added to determine the highest severity grade for the episode. When there are no loose stools for 48 hours, the episode will be considered resolved. The end date for the episode will be the date and time of the last Grade 3, 4, or 5 stool.

For subjects who experience diarrhea, all loose or watery stools will be weighed, graded (for consistency), and evaluated by qualified medical personnel for grossly visible blood. Prior to study start-up, study personnel will receive training and written instructions on how to weigh and evaluate stools for consistency and the presence of blood. If visible blood is noted on a grade 3, 4 or 5 stool, a hemoccult test will be performed to confirm presence of occult blood. Testing may be limited to two samples per day if hemoccult positive.

The assessment of any subject who experiences diarrhea (any Grade 3 – 5 stool) will include a targeted physical assessment and vital signs (temperature, heart rate, blood pressure). Blood pressure will be monitored for diastolic hypotension until diarrhea has resolved (see Table 4). Subjects developing Grade 2 adverse event for diarrhea symptoms also will have urine specific gravity and weight assessed daily until Grade 2 adverse event symptoms have resolved. If any Grade 3 adverse event for diarrhea symptoms occur vital signs will be obtained every 4 hours $\pm$ 1 hour in addition to the procedures listed above. Participants experiencing Grade 3 symptoms or higher illness symptoms may have illness labs collected.

Oral rehydration will be initiated and recorded for subjects with a Grade 2 or higher adverse event of diarrhea, vomiting, dysentery, or dehydration. The hydration of subjects developing diarrhea will be maintained with oral electrolyte solutions (i.e. Pedialyte, Gatorade) by offering at least 1.5 mL for each gram of diarrheal stool lost, as tolerated. Subjects who develop vomiting will be offered at least 1.0 mL for each gram of emesis lost, as tolerated. Subjects will be evaluated by a physician who will consider intravenous hydration, for subjects who:

- cannot tolerate oral fluids,
- experience fluids loss that exceeds their ability to drink replacement fluids,

- have >1000 mL deficit in intake in a 24 hour period,
- experience weight loss of more than 5%,
- have urine specific gravity >1.030 for 12 hours,
- have syncope or near-syncope,
- Grade 3 tachycardia, or
- Grade 3 hypotension.

If the subject is unable to tolerate fluids by mouth and intravenous fluids are deemed necessary by the assessing physician, a 0.9% sterile saline solution 1-liter bolus will be administered intravenously. This bolus will be repeated as needed to resolve Grade 2 and above symptoms at the discretion of assessing physician. Fluid maintenance will be continued using 0.45% NaCl, 5% dextrose, and 20 mEq KCL per liter at a rate of 100mL/hr until subject is able to resume oral rehydration. If the subject develops grade 4 hypotension (see Table 4) despite the aggressive fluid management, the subject will be transferred from the inpatient unit to a hospital setting.

Study day 5

All subjects will be treated (unless meeting criteria for early antibiotic treatment) with a 3-day course of ciprofloxacin 500 mg bid in order to eradicate *S. sonnei* from the intestinal tract of subjects. Trimethoprim (160)-sulfamethoxazole (800) bid x 5 days will be used as alternate for those subjects who cannot tolerate ciprofloxacin.

Criteria for early antibiotic treatment (before study day 5):

- Diarrhea (any severity stool Grade 3 or higher) ***and*** two or more of the following symptoms: severe abdominal pain/cramps, severe nausea, severe headache, severe myalgia, severe arthralgia, gross blood in ≥2 stools, moderate fever (≥38.5ºC), or any vomiting.
- Subjects who experience unexpectedly severe events such as symptomatic hypotension (disproportionate to volume loss), renal dysfunction, or altered mental state (e.g. somnolence) at the discretion of the investigators.
- A study physician determines early treatment is warranted for other reasons.
- Subjects receiving early antibiotic treatment will remain on the unit until the planned day of discharge (day 8)

8.1.5 Study day 8 (Planned discharge)

Subjects will be eligible for discharge from the inpatient unit on day 8 after they have met all of the following criteria:

1. Antibiotic treatment has been initiated on day 5 (or earlier if protocol criteria are met).

2. Two stool samples (collected at least six hours apart) are negative for S. *sonnei*.
3. All symptoms are resolved or resolving.
4. The subject is able to maintain normal hydration status.

Subjects who still have S. *sonnei* isolated from their stool on the planned day of discharge will remain on the unit until they are no longer shedding and all criteria are met. Subjects who meet all discharge criteria and were started on a 5 day course of Trimethoprim instead of the 3-day course of Cipro will receive instructions to take the remaining doses at home as prescribed. Subjects still shedding 48 hours after initiation of ciprofloxacin will be switched to Trimethoprim (1 tablet twice a day for five days).

Following a history-directed physical exam performed by a study investigator and review of concomitant medications, medical history, and a symptom questionnaire to evaluate for reactogenicity, subjects will be discharged with instructions to return for follow-up visits on study days 14±2, 28±2d and 56±4d to provide additional specimens for safety checks or immunology monitoring, described in Section 8.2.2. Subjects will be given an ice-pack and insulated bag for collection of stools at home and subsequent transport of specimens to study site on days 14, and 28,

Discharge Instructions

Subjects will be provided with verbal and written instructions, a memory aid, and digital thermometer to take home and record symptoms and body temperature daily starting at discharge and for the next 5 days. Adverse events that occur during this time will be recorded on and collected from the Memory Aid. Subjects will be instructed to take any remaining doses of study antibiotic per the written schedule provided. Medications (including study antibiotic) taken during this period will also be recorded on and collected from the Memory Aid.

Subjects will be asked to notify the study staff promptly if they develop fever, vomiting, or diarrhea during the 5 days after discharge and may be asked to return to the study site for evaluation.

8.1.6 Early Discharge

If a subject decides to withdraw consent and leave the unit early, he/she will be counseled about the risk of transmission of Shigella to close contacts. Prior to release of the subject, a 1-gram dose of ciprofloxacin will be given to the subject and directly observed that the antibiotic is taken on the unit. This dose of ciprofloxacin has been shown to be 100% effective in eliminating infection and symptoms for treatment of shigellosis not caused by *S. dysenteriae* type 1. The subject will be sent home with four additional 500 mg tablets of ciprofloxacin to complete the 3-day course of therapy at home. He/she will be asked to read and sign the form contained in Appendix C.

Efforts will be made to encourage participants to complete the inpatient stay. Participants who have been released from the inpatient unit before study day 8 will be asked to maintain the study schedule, including return visits for day 10 stool submission and the inpatient

immunogenicity blood draws that they missed to be collected, with the plan to be within 1 day of the scheduled collection. Study staff will attempt to contact subjects by phone to evaluate their well-being.

8.1.7 Study day 14 (±2 day) and 28 (±2 days)

Outpatient visit: On study day 14, subjects will bring a stool specimen for fecal IgA processing, culture, PCR, microbiome. If no stool is available, 2 rectal swabs will be obtained for culture and PCR (fecal IgA only can be done on bulk stool). On study day 28, subjects will bring a stool specimen for microbiome. Subjects will bring their stool specimen within 2- 4 hours of collection (or within 8 hours for stool stored in an insulated bag with an ice pack). On both study days saliva will be collected (cohorts 2-5 only), and blood will be collected for serum IgA, IgG and IgM assays. On day 14, blood will be collected for safety labs and ALS. ALS samples will be processed within 2 hours from the time of collection. A review of the Memory Aid will be performed to evaluate any reactogenic symptoms since discharge from the inpatient unit (including diarrhea, abdominal pain/abdominal cramps, gas, anorexia, nausea, headache, myalgia, malaise, arthralgia, fever, and vomiting). Adverse events, medical history and concomitant medications will be reviewed. Subjects will be given an ice pack and insulated bag on study days 14 for collection of stools at home and subsequent transport of specimens to study site on study days 28.

On day 28, a blood sample for serology (IgA, IgG, and IgM), and memory B-cell assay will be collected from all subjects and a urine pregnancy test (βHCG) will be performed for all female subjects.

Adverse events, medical history, pregnancy, and concomitant medications will be reviewed. History directed physical examinations will be performed as needed prior to discharge from the clinic.

8.1.8 Study day 56 (± 4 days)

Outpatient visit: The final outpatient study visit will be done on study day 56±4. Blood will be collected for serology (IgA, IgG and IgM) and memory B-cell assay. Saliva will be collected (cohorts 2-5 only). A medical interview and history-directed physical examination will also be performed if needed.

Adverse events, medical history, pregnancy, and concomitant medications will be reviewed.

Female subjects who report pregnancy between the time of challenge and the final six month phone call will be followed through delivery or until pregnancy outcome is known.

8.1.9 Study day 180 (± 14 days)

Telephone interview: A telephone interview will occur to query subjects about any medically significant new chronic illnesses, pregnancy, or serious adverse event. As part of the interview,

the ROME III, a questionnaire designed to classify functional gastrointestinal disorders, such as irritable bowel syndrome, will be administered. If subjects report any abnormal symptoms, including new onset of joint pains and arthritis symptoms, then an extra follow up visit may be arranged.

If subjects report any research related injuries or SAEs, they will be followed periodically until the events are resolved or become stable. Follow-up process can be both by telephone and/or asking the subjects to visit the study site, which depends on their conveniences and/or the severity of the particular event.

8.1.10 Unscheduled Visits

If an unscheduled visit occurs, a member of the clinical study team (PI, Co-Investigator, Study Coordinator, or Clinical Nurse) will interview and evaluate the subject to determine the cause of the visit and provide care as needed and information documented on a supplemental visit form. Adverse events and concomitant medications will be reviewed.

8.2 Laboratory Evaluations and Procedures

8.2.1 Laboratory Evaluations (blood volumes are estimates only)

8.2.1.1 Screening (study days -45 to -2)

- Approximately 48 mL blood will be collected:
  - Hematology
    - CBC (WBC, Hgb, platelets, neutrophil and lymphocyte count): (3 mL)
  - Biochemistry
    - Na^+, K^+, Creatinine (BUN will be obtained only if creatinine is above the normal range) Total bilirubin, aspartate aminotransferase/alanine aminotransferase (AST/ALT) (7.5 mL)
    - HLA-B27 (10 ml)
    - HIV, HCV, RPR (syphilis), and HBsAg: (10 mL)
  - Serum βHCG pregnancy test, all females (5mL)
  - Serology
    - anti-*S. sonnei* LPS serum IgG titer: (5 mL SST)
    - Systems biology work (3 x 2.5 mL PAXgene tubes)
- Urine:
  - Urine test for opiates (5 – 10mL urine in sterile screw top container)
- Stool:

- A total of 2 stool samples will be collected for baseline fecal IgA sample between day -5 and prior to receipt of the challenge inoculum on day 0 (challenge not contingent upon results).

Acceptable Laboratory Values for Eligibility and Defining Normal Values

Screening labs may be repeated once if outside the normal limits but considered not clinically significant by the site clinician. Refer to Appendix B for reference ranges.

- WBC and neutrophil counts below the laboratory normal will be allowable if they are in keeping with the levels seen in a condition that is prevalent in our population known as "benign ethnic neutropenia."
- A low creatinine value, a low total bilirubin value, a low ALT value and/or a low AST value are/is acceptable for study inclusion. No lower limits of lab values for creatinine, total bilirubin, ALT, and AST are considered to be clinically significant.

8.2.1.2 Inpatient Baseline (study day -1)

- Approximately 112.5 mL blood will be collected:
  - Serology
    - Antibody (IgG, IgA, IgM) serology assays: (10 mL SST)
  - Cell-based
    - ASC: (50 mL in EDTA tubes)
    - ALS: (10 mL EDTA tubes)
    - Memory B-cells: (30 mL in EDTA tubes)
    - Systems biology work (3 x 2.5 mL PAXgene tubes)
    - Cytokines (5 mL SST tube)
  - βHCG pregnancy test (for all females) ($\geq$5 mL urine in sterile screen top container) will be performed
- Stool: One stool sample, for culture, PCR, fecal IgA testing, baseline microbiome and transcriptome testing will be collected prior to challenge inoculation.
- Saliva (cohorts 2-5 only): A baseline saliva sample will be collected prior to challenge inoculation.

8.2.1.3 Inpatient Study Days (study days 0 – 8)

- **Stool**- Every day, an aliquot of stool will be collected for culture, PCR, microbiome and transcriptomics as detailed in the Schedule of Events, Appendix A. Stool processed in modified buffered glycerol saline (BGS) will be used for quantitative culture and PCR. As scheduled by Appendix A, one Oak ridge tube for fecal IgA (days 0, 3, 7 and 14), 2.0 gm for microbiome and two 0.5 gm aliquots saved in RNAlater for transcriptomics will be processed and kept frozen at -80ºC ± 10ºC. If no stools are passed on a given day, 2 rectal swabs will be collected for culture and PCR.
- **Saliva** samples will be collected (cohorts 2-5 only) on days 1, 3, 5, and 7 and assessed for antigen-specific antibody titers and total IgA concentration.

- **Blood** will be collected on
  - Day 0 (4 hrs. and 12 hrs. post-inoculation) (approximately 25 mL blood) for:
    - Systems Biology (3 x 2.5 mL PAXgene tubes)
    - Cytokines (5 mL SST tube)
  - Day 1 (24 hours post-inoculation) (approximately 12.5 mL blood) for
    - Systems Biology (3 x 2.5 mL PAXgene tubes)
    - Cytokines (5 mL SST tube)
  - Day 3 (approximately 5 mL blood) for
    - Cytokines (5 mL SST tube)
  - Day 5 (approximately 62.5 mL blood will be collected) for:
    - ASC: (50mL in EDTA tubes)
    - Systems biology (3 x 2.5 mL PAXgene tubes)
    - Cytokines (5 mL SST tube)
  - Day 7 (approximately 70 mL blood will be collected) for:
    - Serology
      - Antibody (IgG, IgA, IgM) serology assays: (10 mL SST)
    - Cellular-based:
      - ASC: (50 mL in EDTA tubes)
      - ALS: (10 mL in EDTA tubes)

8.2.1.4 Study day 14±2 day

- **Stool** will be collected for fecal IgA, culture, microbiome, and PCR as described in Section 8.2.2.
- **Blood** (approximately 30.5 mL) will be collected for:
  - Serology
    - Antibody (IgG, IgA, IgM) serology assays: (10 mL SST)
  - Hematology
    - CBC (WBC, Hgb, platelets, and neutrophil and lymphocyte count: (3 mL)
  - Biochemistry
    - Na^+, K^+, BUN/Cr (BUN will be obtained only if creatinine is above normal values in Appendix B), Total bilirubin, aspartate aminotransferase/alanine aminotransferase (AST/ALT) (7.5 mL)
  - Cellular-based
    - ALS: (10 mL – EDTA tube)
- **Saliva** will be collected and assessed for antigen-specific antibody titers and total IgA concentration

8.2.1.5 Study day 28±2d

- **Stool** will be collected for microbiome as described in Section 8.2.2
- **Blood** (approximately 50.5 mL) will be collected:
  - Serology
    - Antibody (IgG, IgA, IgM) serology assays: (10 mL SST)
  - Hematology
    - CBC (WBC, Hgb, platelets, and neutrophil and lymphocyte count: (3 mL EDTA tube)
  - Biochemistry Na^+, K^+, BUN/Cr (BUN will be obtained only if creatinine is above normal values in Appendix B), Total bilirubin , aspartate aminotransferase/alanine aminotransferase (AST/ALT) (7.5 mL)
  - Cellular-based
    - Memory B-cell: (30 mL in EDTA tubes)
- **Saliva** (cohorts 2-5 only) will be collected and assessed for antigen-specific antibody titers and total IgA concentration

8.2.1.6 Study day 56±4d

- Blood (approximately 40 mL) will be collected:
  - Serology
    - Antibody (IgG, IgA, IgM) serology assays: (10 mL SST)
  - Cellular-based
    - Memory B-cell: (30 mL in EDTA tubes)
- **Saliva** (cohorts 2-5 only) will be collected and assessed for antigen-specific antibody titers and total IgA concentration

8.2.2 Laboratory Assays

8.2.2.1 Stool Culture:

The Laboratory for Specialized Clinical Studies (LSCS) at CCHMC will assay for the presence of *S. sonnei*. The first procedure is qualitative and involves streaking swabs containing fecal material to Hektoen Enteric Agar (HEA) plates and will be done at LSCS. The second procedure is quantitative and involves plating serial dilutions of stool suspensions (in BGS) to HEA plates and will be done at WRAIR. All HEA plates will be incubated at 37±1° C overnight. For each subject from the qualitative cultures, up to two blue-green (non-lactose fermenting) colonies will be picked from swab-streaked HEA plate and tested by agglutination by *S. sonnei* polyvalent Group D antiserum at LSCS. Colonies that agglutinate will be recorded as positive for presence of *S. sonnei*. HEA plates used for the serial dilutions that contain blue-green colonies will be processed using a colony blot procedure specific for the detection of *S. sonnei*. The number of positive colonies for each dilution will be recorded and used to calculate the cfu per gram of

stool. These procedures will follow the MOP for culture, isolation and identification for *Shigella sonnei* from stool specimens.

8.2.2.2 Microbiology and Molecular Biology:

2-3 grams of frozen stool in BGS (or a rectal swab, if no bulk stool is collected) will be used for quantitative culture on HEA. Non-lactose fermenting colonies will be tested for the expression of IpaB using a colony immunoblot assay and the presence of the IpaH locus by PCR as described in the MOP. Whole genome sequencing will be performed on selected colonies as described in the MOP.

8.2.2.3 Antibody secreting cells (ASC) and Antibody in Lymphocyte Supernatant (ALS):

The EDTA tubes will be maintained at room temperature and processing (including centrifugation) will begin within 2 hours of collection. Peripheral blood mononuclear cells (PBMC) will be cryopreserved and to minimize variability due to testing on different days, the cells will be assayed in a batch when the complete series of blood collections from a cohort is available. An ELISPOT will be used to enumerate *S. sonnei* LPS. IpaB, IpaC, IpaD and *S. sonnei* Invaplex specific IgA and IgG ASCs per 10^6 PBMC. This procedure will follow the MOP for "Detection of Shigella antigen(s) specific Antibody Secreting Cells (ASC) in Blood of Human and/or Monkey by ELISPOT". The ALS assay will measure the secretion of Shigella-specific antibodies from peripheral blood lymphocytes (PBLs) and will follow the SOP for Purification of $\alpha_4\beta_{7+}$ lymphocytes from frozen peripheral blood mononuclear cells via magnetic separation.

8.2.2.4 Memory B-cell Assay:

The detection of antigen-specific memory B cells induced after challenge with *S. sonnei* 53G will be evaluated in frozen PBMCs collected on day -1 (or any time before challenge on day 0), 28 and 56 by assaying mitogen-expanded PBMCs by ELISPOT for antigen-specific IgA-secreting ASCs using established protocols and the SOP for expansion of human PBMCs to measure *Shigella*-specific IgG and IgA memory B cells by ELISPOT.

8.2.2.5 Serum Immunoglobulins:

Blood (10 mL) will be collected in a tiger top SST on days -1, 7, 14, 28 and 56 for IgA, IgM and IgG assay by ELISA [27-29]. The serum will be separated and stored frozen for determination of specific antibody responses against *S. sonnei* LPS and Invaplex protein-antigens. This procedure will follow the SOP "Enzyme linked immunosorbent assay (ELISA) to *Shigella* antigen-specific serum IgG, IgA and IgM antibodies from human serum samples." Serum collected for serology may also be used for future SBA testing according to the SOP "Bactericidal assay against *Shigella sonnei*."

8.2.2.6 Fecal Secretory IgA:

IgA (total and *S. sonnei* specific) will be determined in fecal extracts using standard ELISA on days -1, 0 (back-up), 3, 7, and 14. The stool sample (4-5gm) will be placed in a 30 mL Oakridge tube and frozen at -75ºC ± 10ºC until ready for the assay. IgA will be recovered from stool samples using a soybean trypsin inhibitor-EDTA procedure. These procedures will follow the

SOP for enzyme linked immunosorbant assay (ELISA) to measure *Shigella* antigen-specific IgG, IgA and IgM antibodies from fecal extract samples, the SOP for enzyme linked immunosorbant assay (ELISA) to measure the total antibody concentration in fecal extract samples, and the SOP for processing of human fecal samples to extract fecal IgA.

8.2.2.7 Salivary IgA:

Saliva collection will follow the schedule as described in Appendix A. Collection of saliva samples will be performed utilizing synthetic oral swabs (Oracol Swab; Malvern Medical). Salivary samples will be collected from all subjects in cohorts 2-5. The subject will gently rub the sponge portion of the Oracol Swab along their gum line for approximately one minute. This procedure has been used in many research studies without untoward risks to subjects [30-32]. There is a minimal risk of choking from or ingesting the swab as it is attached to a handle for ease of use. Some individuals might experience temporary dry mouth. Subjects will be instructed not to eat or drink anything, including chewing gum, for approximately 10 minutes prior to saliva sample collection. Subjects will be instructed to avoid drinking alcohol or using mouthwash for approximately 24 hours and to avoid caffeinated beverages for approximately 12 hours prior to collecting the sample. Saliva samples will be assessed for antigen-specific antibody titers from pre- and post-challenge after adjusting for total IgA. Saliva will be collected and processed according to the MOP.

8.2.2.8 Systems Biology/Microbiomics/Transcriptomics:

Blood will be collected in PAXgene tubes during the pre-screen and on days -1, 0 (4 hrs. and 12 hrs. post-inoculation), 1, and 5. Up to two stool samples for microbiomics and transcriptomics will be collected on days detailed in Appendix A. These samples will be processed and stored as described in the MOP. Analysis of these samples is considered exploratory and will be performed at a future date.

8.2.2.9 Cytokines:

Blood samples will be collected in SST tubes on days -1, 0 (4 hours and 12 hours post-inoculation), 1 (24 hours post-inoculation), 3, and 5. Samples will be frozen at -80°C± 10ºC and stored for future use.

8.3 Concomitant Medications/Treatments:

Any concomitant medication deemed necessary for the welfare of the subject during the inpatient/challenge phase of the study may be given at the discretion of the Investigator. However, it is the responsibility of the Investigator to ensure that details regarding the medication are recorded in full in the subject's source documents and electronic Case Report Forms (eCRFs), including any changes in medication that have occurred during the study.

All medications taken by the subject during 28 days prior to the challenge period will be recorded. All medications taken by a subject during the study (from challenge through Day 56 post challenge) will be recorded. Any change in dose, dosing schedule, or specific medication administered during the study must also be recorded.

In order to avoid the masking of a potential elevated body temperature, prescription and OTC medications that contain acetaminophen, aspirin, ibuprofen, and other non-steroidal anti-inflammatory drugs will not be allowed during the 48 hours prior to challenge administration.

Following investigational challenge administration, the above medications will be allowed if medically required and reported on the eCRF. Medications taken during the outpatient post-challenge phase will be reported by the study subjects.

8.4 Specimen Preparation, Handling, and Shipping

8.4.1 Instructions for Specimen Preparation, Handling, and Storage:

Approximately 456.5 mL of blood (including screening and illness labs) will be collected from each subject over the study. Blood samples will be separated into lymphocyte fractions using a ficoll-hypaque density gradient technique and the lymphocyte fraction saved and frozen pending future testing.

Saliva samples will be collected (cohorts 2-5 only) utilizing synthetic oral swabs for salivary IgA.

Immediately after production, subject will place stool sample for fecal IgA into a sealed container with an ice pack, and brought to the clinic within 8 hours of production. All stool samples will be transported to the LSCS at CCHMC.

All specimens will be labeled with subject code and recorded in specimen tracking log. Subjects will be asked to sign a separate consent form (Sample donation form) to allow the investigators to use their stored specimens in the future. Specimens may be used for research purposes related to shigellosis and other enteric pathogens. Use of these specimens in future studies will require approval by the appropriate Institutional Review Board (IRB). A subject's refusal to grant consent for such use of their stored specimens will not affect his/her eligibility to participate in the current protocol.

Stool specimens will be transported within CCHMC in certified double containers appropriately marked in accordance with laboratory specific operating procedures and approved Institutional Biosafety Committee protocols. Groups of specimens will be transported to the lab in a Styrofoam container with lid and lined with absorbent material.

Laboratory staff that handle shipments have received certified training and certified dangerous good containers must be used. For the laboratory, the laboratory-specific operating procedures and specific Institutional Biosafety Committee approved procedures will be used.

In the isolation facility, standard approved infection control procedures will be used for handling of biohazardous waste and disinfection of the inpatient unit. During stool collections, the remaining stool in the plastic stool collector will be disinfected with bleach for twenty minutes and then flushed into the hopper located in the stool processing area. The disinfected stool collector will then be discarded in hazardous waste.

8.4.2 Study Product Shipment

Live *Shigella sonnei* Product (53G) (lot #1794)

Lyophilized vials of the challenge material (*S. sonnei* 53G, lot# 1794) will be shipped directly to CCHMC prior to the clinical trial. The following procedure is followed by WRAIR PBF for shipment of products, including lyophilized vials of live bacteria, which are stored at -80ºC ± 10ºC:

- Place all frozen packs in a -80ºC ± 10ºC freezer for a minimum of 48 hours before shipment to ensure packs are frozen solid.
- Place dry ice or one layer of freezer packs in the bottom of the shipping container.
- For vials or tubes, place them in a biohazard container and place the biohazard container on top of dry ice or one layer freezer packs (centered, if possible). Ship with a temperature monitor and tape or otherwise secure the temperature monitor in the biohazard container or biohazard bag and activate temperature monitor. Seal the shipping container with shipping tape.
- Place a stamped copy of Form F-040-XX (and Form F-229-XX if requested by PIs and/or CRADA partners) and all paperwork going with the shipment in a plastic bag or envelope and attach it to the top of the shipper lid. Place the appropriate handling labels (e.g., “Freeze Upon Arrival”), on the outside of the shipping box. The QA ICS (Quality Assurance Inventory Control System) must complete the carrier-shipping document with the appropriate information, which must include overnight priority shipping status.

Shigella antigens, negative and positive controls

As detailed in the study MOP, WRAIR will ship specialized Shigella antigens and negative and positive controls directly to the LSCS at CCHMC for training and clinical trial use.

All antigens and control sera will be supplied in 1.0-2.0 mL Nunc® cryovials. The number of vials sent and schedule of shipment will be specified in the MOP.

Microbiological materials

As detailed in the MOP, WRAIR will ship specialized microbiological materials to the LSCS at CCHMC for training and clinical trial use.

9. SAFETY REPORTING AND SAFETY MONITORING

Regulatory requirements including the Food and Drug Administration (FDA) regulations, International Conference on Harmonisation (ICH) Guidelines for Good Clinical Practice (GCP), and European Union (EU) Clinical Trials Directive set forth safety monitoring and reporting responsibilities of study sponsors and investigators to ensure the safety and protection of human subjects participating in clinical trials.

It is the policy of CCHMC that all research involving biohazardous materials will be conducted in a safe manner and in compliance with the *NIH Guidelines for Research Involving Recombinant or Synthetic Nucleic Acid Molecules (NIH Guidelines),* with the CDC's publication *Biosafety in Microbiological and Biomedical Laboratories*, and with all federal, state, local and institutional regulations in order to protect employees, patients, visitors and the greater community and environment at large. The CCHMC IBC shall review all Recombinant DNA Research under its jurisdiction in accordance with these guidelines. CCHMC IBC approval will be received prior to final IRB approval and initiation of this trial.

9.1 Responsibilities

Investigators participating in this clinical trial are responsible for and will:

- Evaluate subject safety including assessment of reactogenic symptoms and AEs for seriousness, severity, and causality
- Immediately (within 24 hours of site awareness) notify the study sponsors of SAEs
- Provide detailed written reports, including necessary documentation requested by the study sponsors or IRB/independent ethics committee (IEC), promptly following immediate initial reports
- Inform the IRB/IEC of AEs as required by applicable regulatory requirements

9.2 Definitions

The investigator is responsible for documentation of AEs according to the detailed guidelines set out below. Subjects will be instructed to contact the investigator immediately should she/he manifest any signs or symptoms of reactogenicity they perceive as serious during the study period as well as immediate reporting (within 24 hours of site awareness) for any new onset symptoms irrespective of severity from Day 0 through Day 56. Approximately six months after the challenge, the subjects will be contacted by phone to document any intervening medically significant new chronic illnesses or serious health events.

9.2.1 Adverse Event (AE)

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

All AEs, solicited or unsolicited, must be graded for severity and relationship to study product (see below).

Solicited Adverse Event

Solicited Adverse Events – reactogenicity events (serious or non-serious) beginning the day of administration of study agent until five days after discharge (recorded per memory aid) from the inpatient unit and include diarrhea, abdominal pain/abdominal cramps, gas, anorexia, nausea, headache, myalgia, malaise, arthralgia, fever, and vomiting. Any unresolved events will be followed to resolution or stabilization.

Unsolicited Adverse Event

Unsolicited Adverse Events- non-reactogenicity events (serious and/or non-serious) occurring from the time of study enrollment to conclusion of study. Any unresolved events will be followed to resolution or stabilization.

FDA defines an AE as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

Adverse events characterized as intermittent require documentation of onset and duration of each episode.

Table 4 Assessment of Solicited Adverse Events

Adverse Event	Severity	Parameter
Fever	1	100.4°F - 101.1°F (38.0-38.4°C)
	2	101.2°F - 102.0°F (38.5-38.9°C)
	3	102.1°F - 104°F (39.0°C - 40°C)
	4	> 104°F (>40°C)
Hypertension (systolic)	1	141-150 mm Hg
	2	151-160 mm Hg
	3	≥161mm Hg
	4	ER visit of hospitalization for malignant hypertension
Hypotension (systolic)	1	85-89 mm Hg
	2	80-84 mm Hg
	3	<80 mm Hg
	4	ER visit or hospitalization for hypotensive shock
Hypotension (diastolic) (with symptoms of dehydration)	1	10 mm Hg decrease upon standing within 3 minutes of sitting or lying supine
	2	20 mm Hg decrease upon standing within 3 minutes of sitting or lying supine
	3	≥30 mm Hg decrease upon standing within 3 minutes of sitting or lying supine

Table 4 Assessment of Solicited Adverse Events		
Adverse Event	**Severity**	**Parameter**
	4	ER visit or hospitalization for hypotensive shock
Bradycardia	1	50-54 bpm*
	2	45-49 bpm
	3	<45 bpm
	4	ER visit or hospitalization for arrhythmia
Tachycardia	1	101-115 bpm
	2	116-130 bpm
	3	>130 bpm
	4	ER visit or hospitalization for arrhythmia
Nausea	1	mild or transient; maintains reasonable intake
	2	moderate discomfort; intake decreased significantly; some activity limited
	3	no significant intake and requires medical intervention
	4	hospitalization required
Abdominal Cramping	1	No interference with daily activities
	2	Some interference with daily activities not requiring medical intervention
	3	Prevents daily activities and requires medical intervention
	4	ER visit or hospitalization
Vomiting	1	1 episode within a 24-hour period
	2	2 episodes within a 24-hour period
	3	>2 episodes within a 24-hour period and requires medical intervention
	4	ER visit or hospitalization for hypotensive shock
Diarrhea**	1	2-3 Grade 3-5 stools (loose or watery) or <400 g/Grade 3-5 (loose or watery) stools per 24 hours
	2	4-5 Grade 3-5 stools (loose or watery) or 400-800 g/ (loose or watery) Grade 3-5 stools per 24 hours
	3	6 or more Grade 3-5 stools(loose or watery) or >800 g/Grade 3-5 (loose or watery) stools per 24 hours and requires medical intervention
	4	ER visit or hospitalization for hypotensive shock

Subjects should be at rest for all vital sign measurements. Oral temperature; no recent hot or cold beverages.
*Not considered Grade 1 bradycardia in conditioned athletes.
**The end of a diarrheal episode occurs when a volunteer does not pass any Grade 3-5 stool within 24 hours.

9.2.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered “serious” if, in the view of either the investigator or study sponsors, it results in any of the following outcomes:

- Death;
- A life-threatening event*;
- Inpatient hospitalization or prolongation of existing hospitalization;
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions; or
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life threatening, or require hospitalizations may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or 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 bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

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

All SAEs must be documented and followed until the event either resolves, subsides, stabilizes, disappears or is otherwise explained, or the subject is lost to follow-up, but not longer than 6 months after the last receipt of test article. Any follow-up activities, with additional or changed information completed on the SAE form, have to be reported within 7 calendar days of receipt of the new information. Clinically significant laboratory abnormalities will be followed up until they have returned to normal or until stable. Reports relative to the subsequent course of an AE noted for any subject must be submitted to the study sponsors. The outcome of SAEs should be assessed in the same manner as all AEs.

Unexpected

An unexpected AE is an adverse reaction, the nature or severity of which is not consistent with the applicable product information (e.g., investigator’s brochure [IB] for an unapproved investigational medicinal product). Unexpected refers to an experience that has not been previously observed. This includes events that are more serious than expected or occur more frequently than expected.

Expected

Any adverse experience, the nature, severity or frequency of which is consistent with the current IB; or with the risk information described in the investigational plan or protocol or consent form.

Procedures to be followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings:

Laboratory test abnormalities will be analyzed based on the grading scale provided in the protocol. Adverse events and SAEs should be documented and reported appropriately.

Adverse events will be followed until resolved or considered stable. Lab values (that are Grade 1 severity) will be followed to resolution or stabilization. Any lab values that are Grade 2 or greater severity will be followed at least until they are Grade 1 severity. AEs may be followed further at the discretion of the site principal investigator or appropriate sub-investigator.

Expedited Safety Report

An expedited safety report is documentation in appropriate form and format summarizing an SAE that meets expedited safety reporting criteria, submitted within the required reporting time frame of applicable regulatory authorities and/or IRBs/IECs of participating countries.

9.3 Safety Reporting Procedures

9.3.1 Reporting Interval

All reactogenicity will be captured and recorded Day 0 through 5 days after discharge from the inpatient unit. All non-serious AEs will be collected Day 0 through Day 56. All SAEs and New Onset Chronic Medical Conditions will be documented from the first administration of study product (Study Day 0) through Study Day 180.

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

At any time after completion of the study, if the investigator becomes aware of an SAE that is suspected to be related to study product, the investigator will report the event to the study sponsors, see Section 9.3.2.

9.3.2 Notification of the Study Sponsors of Serious Adverse Events

All SAEs must be reported immediately (within 24 hours of site awareness) by the investigator, whether or not regarded as possibly attributable to the test articles or antibiotic. Any unexpected fatal or life threatening experience after inoculation with the challenge strain will be reported to the FDA, within 7 calendar days. SAE reports will be provided to the study sponsors, Research Monitor, SMC and the IRBs; contact information provided below. The investigator must report SAEs immediately, within 24 hours of site awareness, by telephone, fax or e-mail (if appropriate) as described in the protocol. This initial notification may include minimal, but sufficient information to permit identification of the reporter, the subject, the test articles, SAEs, and date of onset. The investigator should not wait for additional information to fully document the event before submitting notification. An acknowledgement letter from the sponsor will

confirm the first notification. The report is then to be followed by submission of a completed SAE Report Form provided by the IND-Sponsor as soon as possible, but not more than 3 working days past the initial report, detailing relevant aspects of the SAE in question. All investigator actions and event outcomes must also be reported.

SAE Report Forms are to be used for documentation of these various aspects regarding the event. Hospital records and autopsy reports should be obtained if applicable.

An Independent Research Monitor has been assigned for this study. The independent Research Monitor may discuss the protocol with the investigators, interview subjects, and consult with others outside the study about the research and shall have the authority to stop the protocol, remove subjects from the protocol, and take any necessary steps to protect the safety and well-being of subjects until the IRB can assess the Monitor's Report. The study investigators will consult with the Research Monitor on issues related to subject enrollment and continued participation as needed. The Research Monitor is required to review all unanticipated problems involving risk to subjects, SAEs, and all subject deaths associated with the protocol, and provide an unbiased written report of the event. Reports for unanticipated problems involving risks to subjects or others must be promptly forwarded to the USAMRMC ORP HRPO, in addition to the IRB. At a minimum, the Research Monitor should comment on the event outcomes, and in the case of a SAE or death, comment on the relationship to participation in the study. The Research Monitor should indicate concurrence or non-concurrence with the details of the report provided by the investigator. Reports for events determined by either the investigator or Research Monitor to be related or unrelated to participation and reports of events resulting in death should be promptly forwarded to the IRBs.

Sponsor Notification

For this project, sponsors include funding agencies (PATH and DoD) and the IND-Sponsor (PATH).

The investigator must report all SAEs immediately, within 24 hours of site awareness, to site sponsors whether or not the event is considered related to the study product, and provide to the study sponsors the following information via email (preferred) or telephone:

- Protocol Investigational New Drug (IND) number, investigational product, investigator name and contact number
- Subject identification number
- Serious adverse event, onset date, date of investigational product administration, severity, relationship, and subject's current status

AND Email (preferred) or fax the following documents to the Study Sponsors' Regulatory Affairs office:

- Cover sheet

- Adverse Event Case Report Form
- Supplemental Serious Adverse Event Report Form
- Concomitant Medication Case Report Form or a list of concomitant medications
- Medical record progress notes including pertinent laboratory/diagnostic test results

The investigator will assess all SAEs as being either related or unrelated to the administered product.

When submitting SAE reports via email, the subject line of each email notification may read as follows:

SAFETY REPORT – IND #_____, Protocol Log #_____, Subject ID#_____, Event term

In order to comply with regulations mandating study sponsors notification of specified SAEs to the FDA within 7 calendar days of site awareness, investigators must submit additional information as soon as it is available. The study sponsors will report related, unexpected SAEs to the FDA as specified in 21 CFR.

The investigator must report these additional immediately reportable events (within 24 hours of site awareness) to the study sponsors:

- Any withdrawal of consent during the study
- Pregnancy or intent to become pregnant
- A protocol deviation that jeopardizes the safety of a subject or scientific integrity of the study

IRB Notification

Unanticipated problems involving risk to subjects or others, SAEs related to participation in the study, and all subject deaths will be promptly reported (within 7 calendar days of site awareness) by phone, email, or fax to CCHMC IRB. A complete written report will follow the initial notification.

Investigators are required to forward safety information provided by the study sponsors to the IRB. All SAEs will be reported to the CCHMC IRB according to the appropriate guidelines.

Investigators are required to report adverse events that fit the following criteria within 7 calendar days of the time the investigator becomes aware of them (see below for contact information):

Event is **UNANTICIPATED.** An unanticipated event is any adverse experience where the nature, severity or frequency is not identified in the investigator brochure or described in the protocol. Events which are already cited in the investigator brochure or protocol are not unanticipated and do not have to be reported to the CCHMC IRB

Event is **POSSIBLY RELATED** to the study design, procedures, or drug/device. If the AE is clearly not related to the study drug, device, procedures, or washout process, it would not represent a risk to other subjects in the research and, therefore, does not have to be immediately reported to the CCHMC IRB, but would be reported according to CCHMC IRB guidelines.

CCHMC IRB Contact Information:

Cincinnati Children's Hospital Medical Center (CCHMC) IRB:
3333 Burnet Ave. MLC 7040
Cincinnati, OH 45229
Phone: 513-636-2754
Fax: 513-639-1321
Email: orcra@cchmc.org

Other Notifications

- Independent Research Monitor
- SMC
- FDA (by IND-sponsor) per CBER reporting requirements

9.3.3 Regulatory Reporting for Studies Conducted Under IND

Following notification from the investigator, the IND Sponsor will report any suspected adverse reaction that is both serious and unexpected. The IND Sponsor will report an adverse event as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the drug and the adverse event. The IND Sponsor will notify the FDA and all participating investigators (i.e., all investigators to whom the study sponsors are providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting as specified in 21 CFR Part 312.32. The IND Sponsor will also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information. Relevant follow-up information to an IND safety report will be submitted as soon as the information is available. Upon request from FDA, the IND Sponsor will submit to FDA any additional data or information that the agency deems necessary, as soon as possible, but in no case later than 15 calendar days after receiving the request.

All serious events designated as “not related” to study products will be reported to the FDA at least annually in a summary format.

9.3.4 Reporting of Pregnancy

Subjects will be screened by pregnancy test before participation in this study and instructed to use effective contraception. Sexually active females will have to use birth control (birth control pills, injection hormonal contraceptive, implant hormonal contraceptive, hormonal patch, IUD, sterilization for effective contraceptive methods, spermicidal products and barrier methods are considered acceptable) within two months of challenge and during the entire study.

Although not AEs, pregnancies are reportable events captured through Study Day 180. Pregnancy outcome will be reported (e.g., any premature terminations, elective or therapeutic, and any spontaneous abortions or stillbirths, as well as the health status of the mother and child including date of delivery and infant’s gender and weight). In general, pregnancies should be followed until birth or outcome is known (See Section 9.3.4), although the exact length of follow-up will be determined by each individual product profile and protocol and pending the subject’s permission.

9.4 Investigator’s Assessment of Adverse Events

The determination of seriousness, severity, and causality will be made by an on-site investigator defined as a study clinician licensed to make medical diagnoses and listed on the FDA 1572 (site principal investigator or sub-investigator) who is qualified (licensed) to diagnose AE information, provide a medical evaluation of AEs, and classify AEs based upon medical judgment. This includes but is not limited to physicians, physician assistants, and nurse practitioners.

9.4.1 Assessment of Seriousness

Event seriousness will be determined according to the protocol definition of an SAE (Section 9.2.2).

9.4.2 Assessment of Severity

Adverse events with objective parameters will be graded as listed in Table 4 Section 9.2.1.

Events with subjective parameters will be graded as:

- **Mild (Grade 1)**: No interference with daily activities.
- **Moderate (Grade 2)**: Some interference with daily activities not requiring medical intervention.
- **Severe (Grade 3)**: Prevents daily activities and requires medical intervention.
- **Life threatening (Grade 4)**: ER visit or hospitalization.

A physician will be called to evaluate any subject for intravenous hydration who cannot tolerate oral fluids, experiences fluid loss that exceeds their ability to drink replacement fluids, has >1000 mL deficit in intake in a 24 hour period, experiences weight loss of more than 5%, has a urine specific gravity >1.030 for 12 hours, has syncope or near-syncope, Grade 3 tachycardia, or Grade 3 hypotension.

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

The severity of AEs not specifically defined above will be graded based on the *Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials* dated September 2007 and the NIH comments on the draft guidance (Docket No. 2005D-0155, August 2005). These guidance documents will be utilized to grade the severity of clinically significant laboratory abnormalities with final determination made by the Principal Investigator on a per subject basis.

9.4.3 Assessment of Association

The clinician's assessment of an AE's relationship to the study product is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs will be assessed and documented by clinicians for relationship to study product. The association assessment categories that will be used for this study are:

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

The investigator must provide an assessment of association or relationship of AEs to the study product based on:

- Temporal relationship of the event to the administration of study product
- Whether an alternative etiology has been identified
- Biological plausibility
- Existing therapy and/or concomitant medications

9.4.4 Assessment of Reactogenicity

Reactogenicity events are AEs that are common and known to occur for Shigella and will be collected and graded using a grading scale based on functional assessment or magnitude of reaction. The following reactogenicity events will be solicited beginning the day of administration

of study agent until five days after discharge from the inpatient unit: diarrhea, abdominal pain/abdominal cramps, gas, anorexia, nausea, headache, myalgia, malaise, arthralgia, fever, and vomiting. The grading scales/criteria for these solicited symptoms are listed in Table 4 in Section 9.2.1.

9.5 Safety Monitoring

9.5.1 Safety Monitoring Committee

A Safety Monitoring Committee (SMC) will be established and will be comprised of one Independent Safety Monitor (ISM) and a committee of outside members with expertise in infectious diarrheal diseases and/or clinical trials. The SMC will advise the study sponsors regarding trial safety. The SMC will operate under the rules of a charter that will be written at the organizational meeting of the SMC. At a minimum, the SMC should comment on the safety event and the outcomes of the event or problem. In the case of an SAE or death, the SMC will need to comment on the relationship of the event to participation in the study. The SMC also should indicate whether it concurs with the details of the report provided by the study investigator. Reports for events determined by either the investigator, SMC 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. The SMC and Research Monitor will review available safety data for each cohort prior to making a recommendation on whether or not to proceed to the next cohort. The SMC would recommend one of the following alternatives: advancing to the next dose level, halting, additional subjects at the current dose, or decreasing the current dose. Ad hoc meetings may be required if a halting rule occurs.

9.5.2 Research Monitor

The Research Monitor will function as an independent safety advocate for subjects per 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. The Research Monitor will review available safety data for each cohort prior to making a recommendation on whether or not to proceed to the next cohort. The Research Monitor would recommend one of the following alternatives: advancing to the next dose level, halting, additional subjects at the current dose, or decreasing the current dose. Ad hoc meetings may be required if a halting rule occurs.

An interim safety report will compile findings from the preceding group with the PI's interpretation and plan to go forward. The SMC will review aggregate safety data for increased

rate of occurrence of serious suspected adverse reactions. An interim safety report for each dose level will be prepared and reviewed by the SMC and the Research Monitor. The SMC will convene a meeting or a conference call and provide comments and recommendations before the next higher dose level can proceed. The SMC report and decision will be reviewed and approved by the study sponsors. The SMC report and decision will then be sent to the principal investigator for submission to the IRB. The decision to advance to the next highest dose level will be based solely on the safety profile at the tested dose level.

Criteria for advancing to the next level will include:

- A minimum of 4 weeks has elapsed from the time of the preceding challenge to ensure adequate time for safety monitoring.

Shigellosis is defined as shedding of *S. sonnei* in the stool accompanied by moderate-severe diarrhea (and/or dysentery) along with moderate fever, or one or more severe intestinal symptoms.

The SMC reviewing the data to determine the approximate percentage of subjects who met definition of shigellosis and the primary clinical endpoints as shown below:

  - **Diarrhea**-
    - Moderate- 4-5 loose or watery stools (Grades 3-5) or 400-800 g/Grade 3-5 stools per 24 hours
    - Severe- 6 or more loose or watery stools (Grades 3-5) or >800 g/Grades 3-5 per 24 hours or requires medical intervention
  - **Dysentery**- a Grade 3, 4, or 5 stool with gross blood on at least 2 occasions and reportable constitutional symptoms.
  - **Moderate Fever**- Oral temperature of ≥38.5°C
  - **Symptoms** - one or more severe intestinal symptoms

- If the attack rate (AR) (the percentage of subjects who met clinical endpoints) is less than 60%, the next cohort will receive a higher inoculum, based on deliberations between the investigative team and the SMC.
- ARs at or above 60% may result in verification of the attack rate at the same inoculum dose or a decrease in the inoculum to as low as 100 cfu based on deliberations between the investigative team and the SMC.

9.5.3 Halting Criteria/Rules:

The study will be halted for SMC review/recommendation if:

- Any individual experiences an SAE related to the investigational products
- The same unanticipated severe Grade 3 or greater AEs, such as in safety laboratory data, occurs in two or more subjects. (This does not include two or more subjects

experiencing severe (Grade 3) AEs such as diarrhea, abdominal cramps, fever, or other symptoms included in the clinical definition of shigellosis) determined to be related to the investigational products.

The study sponsors retain the authority to suspend additional enrollment and study interventions/administration of study product for the entire study, as applicable.

10. CLINICAL MANAGEMENT OF EVENTS

10.1 Adverse Event Management

Procedures to be followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings:

Laboratory parameters that are relevant to safety, study outcome measures, and/or clinical outcome will be collected. Abnormal laboratory values based on hematology and clinical chemistry (white blood cell count, hemoglobin, neutrophil and lymphocyte count, platelets, SGOT/AST, SGPT/ALT, total bilirubin, BUN [BUN will be obtained only if creatinine is above normal range], creatinine, sodium, and potassium) after challenge will be considered an AE in accordance with the table of laboratory toxicity grading scale (Appendix B) and should be recorded on the appropriate page of the Case Report Form (CRF).

Adverse events will be followed until resolved or considered stable. Any safety lab values that are Grade 1 severity will be followed to resolution or stabilization. Any safety lab values that are Grade 2 or greater severity will be followed until they are less than Grade 2 severity or followed further at the discretion of the site principal investigator or appropriate sub-investigator.

Additional clinical laboratory evaluations may be performed at other times as required to follow up a serious or SAE or as deemed necessary by the investigator. Non-clinically significant abnormal laboratory values that do not meet adverse event definition(s) will not be recorded as AEs.

Type and Duration of Follow-up of Subjects after Adverse Events

Any research related injuries and AEs would be followed periodically until the events are resolved or become stable. Follow-up process can be both by telephone and/or asking the subjects to visit the study site, which depends on their conveniences and/or the severity of the particular event.

Investigators should follow-up AEs until the final study visit. This may include repeat safety laboratory analysis. For follow-up of SAEs, see Section 9.2.2.

10.1.1 Non-Administration of Study Product in an Individual Subject

An individual may be removed from further study participation or not administered the study product if:

- The subject experiences an SAE unrelated to the investigational product (event will be discussed with the SMC and Research Monitor so as to determine if the event precludes further participation and dosing).
- The investigator deems that stopping the investigational product administration is in the best interest of the subject.
- The subject does not wish to continue with the study.
- The subject is lost to follow-up.

Withdrawal from the trial due to these last two points, if not AE related, does not need to be reported other than as part of routine annual reporting to the FDA and IRBs.

10.1.2 Pregnancy

Female subjects who are found to be pregnant within 180 days after receiving the challenge dose will be followed according to the protocol visit schedule and will also be followed through delivery or until pregnancy outcome is known.

11. CLINICAL MONITORING

11.1 Site Monitoring Plan

Site monitoring is conducted to ensure that the human subject protection, study procedures, laboratory, study intervention administration, and data collection processes are of high quality and meet the study sponsors', ICH E6 and, when appropriate, regulatory guidelines.

Site monitoring for the study will be contracted by the study sponsors. Monitoring visits by a designated clinical monitor 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 and all laboratory results are available. During the site visits, the clinical monitor will review informed consent statements, study-specific MOP, and required regulatory documents. The monitor will verify the completeness and accuracy of the CRFs by comparing them with the source documents. During some of the monitoring visits the actual implementation of study procedures will be observed. A separate monitoring plan document will be developed to describe who will conduct the monitoring, at what frequency monitoring will be done, and what level of detail the monitoring will be conducted. The monitoring plan will include the number of subject charts to be reviewed, which/what proportion of data fields and what will be monitored, who will be responsible for conducting the monitoring visits, and who will be responsible for ensuring that monitoring findings are addressed.

The Investigational Pharmacist (or designated individual) will be responsible for accountability of the challenge product and will document receipt, use, return, or destruction of any unused challenge product. Accountability documentation will be reviewed by a monitor during site monitoring visits.

12. STATISTICAL CONSIDERATIONS

12.1 Study Hypotheses

Hypotheses for primary objectives

1. A human challenge model of *Shigella sonnei* infection using a lyophilized formulation of the challenge strain can be established.
2. We hypothesize an increasing rate of shigellosis with increasing doses of *S. sonnei* 53G and that at least 1 dose level will induce shigellosis in ≥60% of subjects.

Hypotheses for secondary objectives

1. *S. sonnei* 53G will induce a measurable serum or fecal immune response in at least 80% of subjects.
2. Fecal shedding of *S. sonnei* 53G will be detectable by PCR or culture on one or more occasions in at least 80% of subjects.

12.2 Sample Size Considerations

The aim to select a *S. sonnei* strain 53G dose with a ≥ 60% attack rate can be accomplished in 10 subjects with a two-sided exact 95% confidence interval of 26 – 88%. Increasing the number of subjects by 20 for the selected inoculum/fasting regimen (total of 25 subjects at a given inoculum dose) will provide greater confidence (39 - 79%) that the target 60% attack rate will be achieved in future applications of the challenge model.

Follow-up studies evaluating tolerable immunogenic doses will be required with larger numbers of subjects in order to better define the safety profile. All subjects who receive *S. sonnei* 53G will be included in the safety analyses. Safety data, including AEs, physical exam assessments, and laboratory tests will be listed by subject. Adverse events will be coded with the number and proportion of subjects reporting a given event summarized by group. Immune response data will be summarized using similar methodology.

12.2.1 Planned Interim Analyses

There are no planned formal statistical interim analyses. The SMC, along with the Research Monitor will review the data prior to each dose escalation; however, this may not involve any hypothesis testing and will not be considered in estimating the precision of any estimates made at the conclusion of the study.

12.2.2 Analysis Plan

This is a Phase 1 study and as such the analyses will focus on using descriptive techniques to estimate rates, means and their respective confidence intervals. No imputation is planned for missing data.

Rates of local and systemic solicited and unsolicited AEs will be tabulated by level of severity. All rates will be determined with two-sided exact 95% confidence intervals.

During each day of the inpatient period, subjects will be monitored for loose stools (not meeting the diarrhea definition), diarrhea, dysentery, nausea, vomiting, abdominal cramps, fever, headache, abdominal tenderness, abdominal distention or otherwise abnormal abdominal exam.

Descriptive statistics and graphical summaries will be presented.

The planned statistical evaluation is based on the proportion of subjects meeting prospectively defined clinical, microbiological and immunological endpoints. The attack rate will be calculated for all study groups, using the standard definition of: (# with endpoint / # receiving inoculum) x 100%. Summary tables will also be created to detail quantitative and temporal features of the illness such as diarrhea stool frequency and volume, maximum temperature observed, and time to illness and infection. Continuous variables will be analyzed using nonparametric statistics.

Safety: The frequency of symptoms by severity will be calculated, as will frequency and duration of fecal shedding as determined by culture and PCR (exploratory aim). Changes in clinical laboratory values will be considered significant if they are beyond the accepted normal values. Tables will be prepared to list each commonly observed adverse event, the number of subjects 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 and life-threatening) based on the maximum observed severity for each subject.

Immunology: Basic summary statistics as described above will be computed for all immunology endpoints at time points where such data is available. Immunological outcomes will be summarized in a tabular format and graphed to demonstrate kinetics of response. Qualitative (responder rates) and quantitative assessments (log transformed values) will be analyzed. Median increases (fold rises) of antibody concentrations and seroconversion rates will be calculated along with their two-sided exact 95% confidence intervals. Geometric mean titers will also be determined and presented with their two-sided exact 95% confidence intervals. All statistical tests will be interpreted in a two-tailed fashion using an alpha = 0.05.

13. DATA HANDLING/RECORD KEEPING/SOURCE DOCUMENTS

The EMMES Corporation will serve as the Statistical and Data Coordinating Center for this study, and will be responsible for data management, quality review, analysis, and reporting of the study data.

The site principal investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported.

Data collection forms will be derived from the eCRFs to record and maintain data for each subject enrolled in the study. All data collection forms should be completed in a neat, legible manner to ensure accurate interpretation of data. Black or blue ink is required to ensure clarity of reproduced copies. When making a change or correction, cross out the original entry with a single line and initial and date the change. Do not erase, overwrite, or use correction fluid or tape on the original.

Data reported in the eCRF derived from data collection forms should be consistent with the data collection forms or the discrepancies should be explained.

EMMES will provide guidance to investigators on making corrections to the data collection forms and eCRFs.

The site will maintain appropriate medical and research records for this trial, in compliance with ICH E6 (R1), Section 4.9, and regulatory and institutional requirements for the protection of confidentiality of subjects. Authorized representatives of the study sponsors, their designees, and appropriate regulatory agencies will be permitted to examine (and when required by applicable law, to copy) clinical records which include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, and subject files and records kept at the pharmacy, at the laboratories, and medico-technical departments involved in the clinical trial for the purposes of quality assurance reviews, audits, and evaluation of the study safety and progress.

13.1 Data Capture Methods

Clinical data (including AEs, concomitant medications, and reactogenicity data), clinical assessments, (including reported symptoms by the subject, assessment of stool, vital signs, and physical examinations), and clinical laboratory data (including safety labs) will be entered into a computerized data entry system. The data system includes password protection and internal quality checks to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents.

13.1.1 Types of Data

Data for this study will include safety, laboratory (immunologic, microbiologic, and PCR), and outcome measures (e.g., reactogenicity, immunogenicity, microbiology). Interim reports will include the rate of accrual, including demographics (e.g. age, race, and ethnicity), as well as reports of all SAEs. Data will be locked when all data queries have been completed. Final analysis will await completion of the study by all subjects and the locking of the database.

13.2 Study Records Retention

Study documents will be retained for a minimum of 2 years after the last approval of a marketing application and until there are no pending or contemplated marketing applications, or when at least 2 years have elapsed since the formal discontinuation of clinical development of an investigational product. These documents will be retained for a longer period, however, if required by local regulations. No record will be destroyed without the written consent of the study sponsors.

CCHMC will maintain appropriate medical and research records for this trial, in compliance with ICH E6, Section 4.9, regulatory and institutional requirements for the protection of confidentiality of subjects. CCHMC will permit authorized representatives of the study sponsors and regulatory agencies to examine (and when required by applicable law, copy) clinical records for the purposes of clinical site monitoring, quality assurance reviews, audits, and evaluation of the study safety and progress.

It is the policy of the US Army Medical Research and Materiel Command (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 data base includes the subject's name, address, and Social Security Number; study title; and dates of participation. The intent of this data base 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 to provide 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. The Form 60-R will be mailed to USAMRMC for storage.

13.3 Source Documents

The site will maintain appropriate medical and research records for this trial, in compliance with ICH E6 (R1) GCP, Section 4.9, and regulatory and institutional requirements for the protection of confidentiality of study participants. The site will permit authorized representatives of the study sponsors and regulatory agencies to examine clinical records for the purpose of quality assurance reviews, audits, and evaluation of the study safety and progress.

Study documentation will be coded and stored in locked cabinets in work areas that are accessible by badge only. Electronic study data will be coded and stored on computers that are accessible only by password and will be maintained in work areas that are accessible by badge only.

Source data are all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source documentation include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, documentation of phone collection of study participants' memory

aid information, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, X-rays and study subject files and records kept at the pharmacy.

13.4 Protocol Deviations

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or Manual of Procedures requirements. The noncompliance may be either on the part of the subject, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly. Deviations from the protocol will be reported promptly to any Institutional Review Boards responsible for the study.

These practices are consistent with GCP:

21 CFR 50 and 56 and, as applicable, ICH6,

4.5 Compliance with Protocol, Sections 4.5.1, 4.5.2, and 4.5.3,

5.1 Quality Assurance and Quality Control, Section 5.1.1, and 5.2.0 Noncompliance, Sections 5.2.1, and 5.2.2.

It is the responsibility of the site to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations must be promptly reported to the study sponsors.

All deviations from the protocol must be addressed in study subject source documents. A completed copy of Deviation Form must be maintained in the Regulatory File as well as in the subject's source documents. Protocol deviations must be sent to the local IRB/IEC per their guidelines. The site PI/study personnel is responsible for knowing and adhering to their IRB requirements.

14. QUALITY CONTROL AND QUALITY ASSURANCE

The site is responsible for conducting routine quality assurance (QA) and quality control (QC) activities to internally monitor study progress and protocol compliance. The Principal Investigator will provide direct access to all trial-related sites, source data/documents, and reports for the purpose of monitoring and auditing by the study sponsors, and inspection by local and regulatory authorities. The Principal Investigator will ensure all study personnel are appropriately trained and applicable documentations are maintained on site.

Clinical monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirements. Clinical monitoring reports will be submitted to the study sponsors.

15. ETHICS/PROTECTION OF HUMAN SUBJECTS

15.1 Ethical Standard

The investigator will conduct the study in full conformity with the principles set forth in The Belmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the US National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR Part 46 and 21 CFR 50 and 56 and the ICH E6; 62 Federal Regulations 25691 (1997).

15.2 Institutional Review Board

CCHMC will provide for the review and approval of this protocol and the associated ICFs by their IRB located at CCHMC, 3333 Burnet Avenue, MLC 5020, Cincinnati, Ohio, 45229-3039, under the institution's US Federal wide Assurance. Any amendments to the protocol or consent materials must also be approved before they are placed into use. Only those IRB members who are independent of the investigators and the study sponsors should provide an opinion on study-related matters. No deviations from or changes to the protocol will be initiated without prior approval of an appropriate amendment.

16. INFORMED CONSENT PROCESS

The informed consent process will be conducted on the first screening day in a private room at CCHMC. The consent will explain that subjects may withdraw consent at any time throughout the course of the trial. Extensive explanation and discussion of risks and possible benefits of this investigation will be provided to the subjects in understandable language. Study investigators or appropriately qualified study personnel who have been delegated authority by the study investigator will explain the purpose and the details of the protocol to the participant. An illiterate subject will not be enrolled for subject recruitment. Participants are required to read the consent form, fully discuss their concerns and ask questions until their complete satisfaction. They will be allowed to take their time before making a decision or take the form home for consideration and consultation with their family. Participants and clinical staff must sign and date the consent form. The original of the signed consent form will be filed in the participant's source document, and a copy will be provided to the participant.

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

To ensure comprehension of the study, on the day of admission to the inpatient unit, a written test will be administered which the participants will have 2 chances to pass successfully. If subjects are unable to score 70% or greater on the test, they will be excluded from the study.

16.1 Subject Confidentiality

Each subject will be assigned a unique study identifier. All data collection sheets will identify the subject by a unique identifier, and the date. Names will not be used on any samples or in any publication of this study. All efforts will be made to protect the privacy of subjects.

Subject confidentiality is strictly held in trust by the participating investigators, their staff, and the study sponsors and their agents. This confidentiality includes documentation, investigation data, subject's clinical information, and all other information generated during participation in the study.

This information and data will not be used by the site principal investigator or other study personnel for any purpose other than conducting the study. These restrictions do not apply to: (1) information which becomes publicly available through no fault of the site principal investigator or other study personnel; (2) information which is necessary to disclose in confidence to an IRB solely for the evaluation of the study; (3) information which it is necessary to disclose in order to provide appropriate medical care to a study subject; or (4) study results which may be published.

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

No information concerning the study or the data generated from the study will be released to any unauthorized third party without prior written approval of the study sponsors and the subject.

16.2 Study Discontinuation

Each subject has the right to withdraw from the trial at any time for any reason without affecting their right to treatment by the investigator. The investigator also has the right to withdraw the subjects in the event of intercurrent illnesses, AEs, a subject's failure to comply with study procedures, or if it is determined to be in the subject's best interest. If a subject withdraws for safety reasons, an AE or SAE will be reported as described in Sections 6.1.

If a subject withdraws for other reasons, the withdrawal will be reported as part of the annual review process. If a subject chooses to withdraw from the study after challenge but before the time of planned administration of antibiotics, they will be asked to inform either the PI for the trial or an Associate investigator of the study and antibiotic therapy will be initiated. They will be encouraged to remain on the Inpatient Unit until a three-day course of treatment can be completed. If they choose not to; the study staff will observe the first dose of antibiotic and instruct the subjects how to complete the antibiotic therapy. They will also be encouraged to complete all remaining outpatient visits for the trial as described in Section 6.1 and Appendix A.

16.3 Future Use of Stored Specimens

Subjects will be asked to provide consent to allow the investigators to use their stored specimens in the future. Specimens will be used for research purposes related to the development of improved vaccines for shigellosis and other enteric pathogens at CCHMC or other external collaborative institutions. If the investigators have access to identifiers, then prior to future use of the samples, appropriate IRB approval will be obtained. Samples may be shared in a de-identified manner with non-study investigators and the level of IRB review will be discussed with the appropriate IRB prior to initiation of the study. A subject's refusal to grant consent for such use of their stored specimens will not affect his/her eligibility to participate in the current protocol. Specimens will be stored in the de-identified state in which they were originally labeled after collection and stored in a secure freezer at CCHMC for 20 years. If samples still remain at the end of the 20 years of storage, they will be destroyed. Only study investigators or designated persons by the principle investigators will have access to the stored specimens.

16.4 Research Related Injuries

All study-related medical care will be provided to subjects without cost. Should a subject be injured as a direct result of participating in this research project, s/he will be provided medical care by the staff of a military-affiliated medical center at no cost to the subjects, for that injury. The subjects will not receive any injury compensation, only medical care. The subjects will not be compensated for care if s/he chooses to seek care from his/her own physician.

If a subject is injured because of participation in this research and is a DoD healthcare 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 healthcare system, as long as the subject remains a DoD healthcare beneficiary. This care includes, but is not limited to, free medical care at DoD hospitals or clinics.

During the challenge phase, subjects who require medical treatment beyond what can be provided safely on the inpatient unit at CCHMC will be transferred to a local full-service hospital for care. In the event this occurs, the study sponsors agrees to reimburse the hospital for all reasonable expenses incurred by that hospital in providing medical treatment and/or hospitalization reasonably necessary to address the subject's medical needs. The Cincinnati Children's Hospital has no plan to provide compensation to subjects if they experience injury or other bad effects which are not the fault of the study doctors. Subjects will only be treated for injuries that are directly caused by the research study. In the event this occurs, the study sponsors agree to reimburse the Hospital for all reasonable expenses incurred by the Hospital in providing medical treatment and/or hospitalization reasonably necessary to address any injury to a Subject that, in the reasonable judgment of Hospital and study sponsors, occurs directly as a result of the administration of the IMPs or performance of study procedures in accordance with the Protocol, but only to the extent such expenses are not:

- the result of a foreseeable side effect as indicated in the Protocol

- reimbursed by (or submitted for reimbursement to) the Subject's insurance or any governmental program or other third-party payer providing medical or hospital coverage; provided, however, that this provision shall not obligate Hospital to submit such costs to the prospective Subject's insurance or any governmental program or other third-party payer coverage
- attributable to a failure of Hospital, or any of the Investigator Personnel, including PI, to adhere to the terms of the Protocol, study Sponsors' written instructions or Applicable Law
- attributable to the negligence, recklessness or willful misconduct or omission of Hospital or any of its Investigator Personnel, including PI
- attributable to a pre-existing abnormal medical condition or underlying disease of the Subject or treatment that would have been provided to the Subject in the ordinary course notwithstanding participation in the study, or
- attributable to the failure of the Subject to follow the reasonable instructions of Investigator Personnel or Subject's physician.

Transportation to and from military hospitals or clinics will not be provided. No reimbursement is available if the subject incurs medical expenses to treat research-related injuries from outside or private providers. No compensation is available for research-related injuries. 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 United States 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 ICF and will be discussed with the subject by the investigator or designee before the subject signs the ICF to participate in the study.

17. PUBLICATION POLICY

Following completion of the study, the investigator is expected to publish the results of this research in a scientific journal. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov [23], which is sponsored by the National Library of Medicine. Other biomedical journals are considering adopting similar policies. It is the responsibility of the sponsor to register this trial in an acceptable registry. Any clinical trial starting enrollment after 01 July 2005

must be registered on or before patient enrollment. For trials that began enrollment prior to this date, the ICMJE member journals will require registration by 13 September 2005, before considering the results of the trial for publication.

The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., Phase 1 trials), would be exempt from this policy.

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19. APPENDICES

19.1 Appendix A: Schedule of Events – for each Cohort

Study Event	Screening Day -45	Day -1	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 14	Day 28	Day 56	Day 180
Visit Number	**00A**	**00B**	**01**	**01A**	**01B**	**01C**	**01D**	**01E**	**01F**	**01G**	**01H**	**O2**	**O3**	**O4**	**O5**
Visit Window	**-45 to -2**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**±2**	**±2**	**± 4**	**±14**
Facility	**Outpatient**	**Inpatient**										**Outpatient**			
Inpatient Period		X	X	X	X	X	X	X	X	X	X				
Review of Inclusion /Exclusion Criteria	X	X	X												
Sign Informed Consent	X														
Complete Written Test[1]		X													
Medical interview[2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination[3]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Vital Signs [4]	X	X	X	X	X	X	X	X	X	X	X				
Hematology[5]	X											X	X		
Biochemistry[6]	X											X	X		
Hepatitis B, C, HIV and syphilis[7]	X														
Urine for Opiates	X														
Serum Pregnancy Test[8]	X														
Urine Pregnancy Test		X											X		
HLA B27 antigen[9]	X														
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Review Medical History	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Reactogenicity[10]			X	X	X	X	X	X	X	X	X	X			
Collection of AEs			X	X	X	X	X	X	X	X	X	X	X	X	
Collections of SAEs, significant new chronic illnesses, pregnancy			X	X	X	X	X	X	X	X	X	X	X	X	X
Serology[11]	X	X								X		X	X	X	
Stool culture for Shigella		X	X	X	X	X	X	X	X	X	X	X			

Study Event	Screening Day -45	Day -1	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 14	Day 28	Day 56	Day 180
Visit Number	**00A**	**00B**	**01**	**01A**	**01B**	**01C**	**01D**	**01E**	**01F**	**01G**	**01H**	**O2**	**O3**	**O4**	**O5**
Visit Windows	**-45 to -2**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**N/A**	**±2**	**±2**	**± 4**	**±14**
Facility	**Outpatient**	**Inpatient**										**Outpatient**			
Challenge			X												
Stool grading[12]			X	X	X	X	X	X	X	X	X				
Stool for IgA[13]		X	X			X				X		X			
Stool for PCR		X	X	X	X	X	X	X	X	X	X	X			
Stool for Microbiome		X	X	X	X	X	X	X	X	X	X	X	X		
Stool for transcriptomics		X	X	X	X	X	X	X	X	X	X				
Blood for Transcriptome (systems biology)[14]	X	X	X	X				X							
Antibody secreting cells (ASC)[15]		X						X		X					
Antibody lymphocyte supernatant (ALS)[16]		X								X		X			
Memory B-Cell[17]		X											X	X	
Cytokines[18]		X	X	X		X		X							
Salivary IgA[19]		X[20]		X		X		X		X		X	X	X	
Start antibiotic therapy[21]								X							
Planned discharge[22]											X				
Study completion															X
Post-study safety assessment (via telephone follow-up)															X
Total Blood volume (mL)[23]	48	112.5	25	12.5		5		62.5		70		30.5	50.5	40	

[1] Sample test is provided to participants at screening and administered on day -1

[2] The medical interview during screening is to assess eligibility. During the inpatient and outpatient period, the interview will be to update interim medical history, monitor safety, and confirm ongoing eligibility.

[3] A complete physical will be done for the initial screening visit and admission to the inpatient unit. All other physical exams will be history directed.

[4] Vital signs (temperature, pulse, and blood pressure) will be obtained at screening and at least daily during inpatient study (more frequently if indicated per Management and Assessment of Diarrhea, Vomiting, Dysentery, and Dehydration guidelines in section 8.1.4).

[5] Hematology labs (CBC) include WBC, hemoglobin, neutrophil and lymphocyte count, and platelets (3 mL) (and as indicated per Management and Assessment of Diarrhea, Vomiting, Dysentery, and Dehydration guidelines in section 8.1.4).

[6] Biochemistry will include sodium, potassium, creatinine, total bilirubin, ALT and AST and A BUN will be obtained only if Creatinine is > 1.5. (7.5 ml) (Also obtain as indicated per Management and Assessment of Diarrhea, Vomiting, Dysentery, and Dehydration in section 8.1.4).

[7] Hepatitis B, C, HIV and syphilis (10mL)

[8] Serum pregnancy test (5 mL)
[9] HLA B27 antigen (10 ml)
[10] Reactogenicity will be recorded on the memory aid starting on day of discharge and for the next 5 days.
[11] Serology (anti-*S.sonnei* LPS serum IgG) (5mL screening), (10 mL Day -1 to day 56); serum collected for serology may also be used for SBA testing
[12] Fecal samples will be collected post-challenge through day of discharge for assessment of diarrhea and dysentery. A rectal swab will be obtained for culture and PCR if subject unable to provide a stool sample by midnight each day.
[13] Two stool samples will be collected between Day -5 and time of challenge on Day 0 for fecal IgA. Challenge is not contingent upon the results.
[14] Transcriptomics (systems biology) - 3 x 2.5 mL PAXgene tubes. Blood for transcriptomics and cytokines on Day 0 will be done at 4 hours and 12 hours post-inoculation and 24 hours post –inoculation on Day 1.
[15] ASC=(50 mL in EDTA tubes); cells collected for ASC may also be used for magnetic separation testing
[16] ALS=(10 mL EDTA tubes); cells collected for ALS may also be used for magnetic separation testing
[17] Memory B-cells=30mL in EDTA tubes
[18] Cytokines = 5 mL SST tubes (4 hours and 12 hours post inoculation), day 1 (24 hours post –inoculum), days 3, 5
[19] Saliva samples will be collected in cohorts 2-5 only.
[20] A baseline saliva sample will be collected between day -1 and any time before challenge on day 0.
[21] Ciprofloxacin 500 mg BID x 3 days or (alternate) trimethoprim/sulfamethoxazole 160/800mg BID x 5 days. Subjects may be treated earlier if they meet the criteria for early antibiotic treatment. Subjects will complete antibiotic treatments at home if they meet discharge criteria before completing the treatment period.
[22] Subjects will be discharged upon meeting the discharge criteria, which may be beyond day 8. Subjects remaining on the inpatient unit past day 8 will continue to have daily evaluations until meeting discharge criteria.
[23] Approximate total blood volume to be collected at each visit.

19.2 Appendix B: Toxicity Grading

During screening, subjects will have blood drawn to determine if any clinical laboratory abnormalities exist that would preclude study participation. The clinical toxicity grading scale below is based on the FDA Guidance for Industry dated September 2007. In the case where the institutional normal parameters of the laboratory used for this study vary from the FDA Guidance, the institutional normal parameters were inserted in the table and will serve as the standard against which eligibility and adverse event reporting will be based.

Toxicity grading of safety screening laboratory chemistry and blood-count results

Laboratory	Normal Range	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening *
Sodium, low, mEq/L	136-145	130-135	123-129	116-122	< 116 or abnormal sodium with mental status changes or seizures
Sodium, high, mEq/L	136-145	146-150	151-157	158-165	> 165 or abnormal sodium with mental status changes or seizures
Potassium, high, mEq/L	3.5-5.1	5. 2-6.0	6.1-6.5	6.6-7.0	> 7.0 or abnormal potassium with life-threatening arrhythmia
Potassium, low, mEq/L	3.5-5.1	3.0-3.4	2.5-2.9	2.0-2.4	< 2.0 or abnormal potassium with paresis, ileus or life-threatening arrhythmia
Blood Urea Nitrogen (BUN) mg/dL	7-20	>1.0-2.5 x ULN**	2.6-5.0 x ULN**	5.1–10 x ULN**	>10 x ULN**
Creatinine	Male - ≤1.17 Female- ≤0.95	>1.0–1.5 x ULN**	>1.5–3.0 x ULN**	>3.0–6.0 x ULN**	> 6.0 x ULN** or dialysis required
Liver Function Tests ((ALT, AST),) increase by factor	ALT – 1.1x<78 AST – 1.1x<37	>1.0-2.5 x ULN**	>2.5-5.0 x ULN**	>5.0-10 x ULN**	> 10 x ULN**

Total bilirubin – when accompanied by any increase in Liver Function Test; increase by factor	0.1-1.1	>1.0-1.25 x ULN**	1.26-1.5 x ULN**	1.51-1.75 x ULN**	> 1.75 x ULN**
Hgb (female), g/dL	11.7-15.7	11.0-11.6	9.5 – 10.9	8.0-9.4	< 8.0
Hgb (male), g/dL	13.0-17.7	12.5-13.2	10.5-12.4	8.5-10.4	<8.5
WBC, increase, cells, x 10^3u/L	4.5-11	>11.0 – ≤15.0	>15 - ≤ 20	>20.0 – ≤25.0	>25.0
WBC, decrease, cells, x 10^3u/L	4.5-11	2.5-<4.5	1.5-<2.5	1.0-<1.5	<1.0
Absolute Neutrophil Count, x 10^3u/L	1.5-7.7	1.5-<1.8	1.0-<1.5	0.5-<1.0	<0.5
Lymphocytes, x 10^3u/L	1.0-4.8	0.75-<1.0	0.5-<0.75	0.25-<0.5	<0.25
Platelets, decrease, cells/mm^3	134-466	125,000-<135,000	100,000-124,000	25,000-99,000	< 25,000
Urine protein	Trace	1+	2+	≥3+	Hospitalization or dialysis
Urine glucose	0	1+	2+	≥3+,	Hospitalization for hyperglycemia
Urine blood(non-menstruating)	0-1	3-10	11-50	>50 and/or gross blood	Hospitalization or packed red blood cells (PRBC) transfusion

* The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening. For example, a low sodium value that falls within a grade 3 parameter (116-122 mE/L) should be recorded as a life-threatening hyponatremia event if the subject had a new seizure associated with the low sodium value.

** ULN is upper limit of normal in lab.

In addition, please note that the toxicity grading scale outlined in the FDA Guidance for Industry dated September, 2007 will also be used as reference values for grading laboratory abnormalities encountered during the trial (see Appendix B). However AEs, guided by the Appendix B, will be graded for severity based on comparisons to subject baseline values, presence of clinical symptoms and an assessment by the principal investigator.

19.3 Appendix C: Information Form if a Subject Prematurely Withdraws from the Trial

Safety and Infectivity of an Oral Challenge with *S. sonnei* 53G

I,_____, am withdrawing consent from the Shigella challenge study. I have been told that I am leaving the inpatient unit before I have passed two stool specimens that are negative for Shigella.

I have been advised by Dr. Frenck that the Shigella strain could still be living in my intestines and could be passed to my close contacts, who could develop symptoms of Shigella infection (diarrhea, blood in stool, fever). If one of my close contacts develops diarrhea, I have been told that he/she may come to the Gamble Program Outpatient Clinic for a clinical evaluation and testing of his/her stool specimen free of charge.

I have been told that each time I use the bathroom I should wash my hands well with soap and water, and dry them completely. I have been advised that I should continue these precautions until my final stool specimens have been shown to be negative for Shigella.

I have been told that I should complete my antibiotic treatment at home to eliminate the Shigella from my intestine. Even though I am leaving the unit early, I have been told that it is very important for me to come to my outpatient follow-up visits to the Gamble Program Outpatient Unit to have my stools checked for the Shigella and to provide samples, according to the schedule that I have been given. I will receive a signed copy of this document and a copy will remain on in my study file.

_____ _____
Signature of Subject Date

_____
Printed Name of Subject

_____ _____
Signature of Witness Date

_____
Printed Name of Witness

_____ _____
Signature of Principal Investigator Date