Clinical Study Protocol

Protocol Title:	**Controlled Human Infection Model Challenge/Rechallenge: *Shigella flexneri* 2a and *S. sonnei* cross-protective antigens discovery in healthy adults in the United States**
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Investigator's Agreement

Controlled Human Infection Model Challenge/Rechallenge: *Shigella flexneri* 2a and *S. sonnei* cross-protective antigens discovery in healthy adults in the United States

"I have read this protocol and agree to conduct the study as outlined herein in accordance with International Conference on Harmonization Good Clinical Practice Guideline and FDA and DoD Regulations."

_____ _____

Kawsar R. Talaat, MD Date
Principal Investigator

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II. ABBREVIATIONS

Abbreviation	Definition
ADi	Antigen Discovery, Inc
AE	Adverse event
AESI	Adverse event of special interest
ALT	Alanine aminotransferase
ALS	Antibody from lymphocytes supernatant
ASC	Antigen stimulated cells
BMI	Body mass index
BP	Blood pressure
BPR	Batch Production Record
BUN	Blood urea nitrogen
CBC	Complete blood count
CFU	Colony forming units
cGMP	Current Good Manufacturing Practice
CHIM	Controlled Human Infection Model
CIR	Center for Immunization Research, Johns Hopkins School of Public Health
CLIA	Clinical Laboratory Improvement Amendments of 1988
CR	Congo red
eCRF	Electronic Case Report Form
CRF	Case Report Form
CVIA	Center for Vaccine Innovation and Access
DMP	Data management plan
DoD	Department of Defense
DSMB	Data safety monitoring board
EDC	Electronic data capture
ELISA	Enzyme-linked immunosorbent assay
GCP	Good Clinical Practice
h	Hours
H2 blockers	H2 receptor agonists
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HIV-1	Human Immunodeficiency Virus 1
HLA	Human leukocyte antigen
IATA	International Air Transport Association
ICF	Informed Consent Form

ICH	International Conference on Harmonisation
IgG; IgA	Immunoglobulin G; Immunoglobulin A
IP	Investigational Product
IRB	Institutional Review Board
IV	Intravenous
JHSPH	Johns Hopkins Bloomberg School of Public Health
JHU	Johns Hopkins University
JHH	Johns Hopkins Hospital
LPS	Lipopolysaccharide
MCB	Master cell bank
mL	Milliliter
mm	Millimeter
MOP	Manual of procedures
NMRC	Naval Medical Research Center
ORS	Oral rehydration solution
PCB	Production cell bank
PBF	Pilot Bioproduction Facility (WRAIR)
PBMC	Peripheral blood mononuclear cells
PBS	Phosphate buffered saline
PE	Physical Exam
PI	Principal Investigator
PI-IBS	Post-infectious irritable bowel syndrome
PSRT	Protocol Safety Review Team
PVT	Psychomotor vigilance test
QA	Quality assurance
QC	Quality control
RNA	Ribonucleic acid
SAE	Serious adverse event
SAP	Statistical analysis plan
SBA	Serum bactericidal antibody
SMP	Safety Monitoring Plan
SOP	Standard Operating Procedure
SSP	Study Specific Procedure
SWI	Sterile water for injection
TD	Travelers' diarrhea
TMF	Trial Master File
TSA	Trypticase soy agar

TSB	Tryptic soy broth
VS	Vital signs
WBC	White blood cell
WRAIR	Walter Reed Army Institute of Research

III. CLINICAL PROTOCOL SYNOPSIS

Protocol Title	**Controlled Human Infection Model Challenge/Rechallenge: *Shigella flexneri* 2a and *S. sonnei* cross-protective antigens discovery in healthy adults in the United States**
Name of the investigational products	• *Shigella flexneri* 2a 2457T (lot 1617) • *Shigella sonnei* 53G (lot 1794)
Study Site	Johns Hopkins Center for Immunization Research
Principal Investigator	Kawsar Talaat, M.D.
Study Hypothesis	Challenge with one species of *Shigella* (*Shigella flexneri* 2a or *Shigella sonnei*) will induce some cross-protective immunity such that a subset of volunteers will be protected upon challenge with the second strain.
Study Rationale	The goal of this study is to better understand *Shigella* immunity. The study will compare the shigellosis rate in subjects previously challenged with a heterologous *Shigella* serotype to the attack rate in naïve subjects. Subsequent evaluations of the immune response will seek to identify cross-protective *Shigella* antigens which could improve upon the current requirement for serotype-specific O-antigens from the most common *Shigella* species.
Study Population	Up to 54 healthy participants (male or non-pregnant, non-nursing female), aged 18-50 years (all inclusive) from the Baltimore, MD-Washington, DC region and adjacent regions.
Study Design	This is a single-center controlled human infection model (CHIM) study designed to assess the cross-protective efficacy and markers of protection after challenge and heterologous rechallenge with *Shigella* strains of different serotypes in naïve healthy adult volunteers. As shown in Table S1, a group of up to 13 naïve participants will be challenged with 1500 colony forming units (cfu) of *S. sonnei* 53G (Group 1A1). At least 3 months later, 11 of these volunteers, who are willing and eligible for rechallenge, along with up to 8 newly recruited naïve participants, will be challenged with 1500 cfu of *S. flexneri* 2a (Group 1B1). Additionally, nine newly recruited naïve participants will be challenged with 1500 cfu of *S. sonnei* 53G (Group 1A2) and later 8 of these volunteers willing and eligible for rechallenge will be rechallenged with 1500 cfu of S. flexneri 2a (Group 1B2). An additional group of 13 naive participants will be challenged with 1500 cfu of S. flexneri 2a (Group 2A1). At least 3 months later, up to 11 of those volunteers from Group 2A1 willing and eligible to proceed to another challenge, along with 5 or more naïve participants, will be challenged with 1500 cfu of S. sonnei 53G (Group 2B1), and another group of up to 6 naive participants will be challenged with 1500 cfu of S. flexneri 2a (Group 2A2). Each volunteer will be followed for 6 months from the time of their last challenge. Table S1. Clinical trial study design.

Group/cohort	N	Sub-group	N naïve	N veterans	Challenge strain (1500 cfu/dose)
1A	13	1	13	0	*S. sonnei* 53G
	9	2	9	0	*S. sonnei* 53G
1B	19	1	Up to 8	11 from 1A1	*S. flexneri* 2a 2457T
	8	2	0	8 from 1A2	*S. flexneri* 2a 2457T
2A	13	1	13	0	*S. flexneri* 2a 2457T
	Up to 6	2	Up to 6	0	*S. flexneri* 2a 2457T
2B	Up to 16	1	5	Up to 11 from 2A1	*S. sonnei* 53G

Breakdown of study cohorts/groups: Each cohort is defined by which challenge agent is administered first (e.g., Cohort 1, *S. sonnei*, Cohort 2, *S. flexneri* 2a). The groups refer to the admission; Group A is the first admission/challenge per cohort, B, the second admission/rechallenge. Within the A groups, all volunteers are naïve; within each of the B groups, up to 20 volunteers will be veterans from the A groups and up to10 volunteers will be naïve. The numbers after the letter e.g. 1A1, denote subgroups that occurred at different admissions.

Clinical Monitoring

After challenge, participants will be monitored for shigellosis by daily medical checks, vital sign determinations, and grading and weighing of all stools. Monitoring for fecal shedding of the challenge strain will occur daily after challenge by standard microbiological and molecular assays (detailed description given in separate study specific procedure [SSP]). If a participant is unable to provide a stool sample by 1300 hours, s/he will be asked to obtain a rectal swab. Swabs will be used starting the day after challenge.

Mucosal and systemic antibody responses to the challenge strains will be assessed throughout the study, as shown in the schedule of events.

Antibiotic Treatment

Routine antibiotic treatment with antibiotics to which the strains are susceptible will commence for all participants approximately 120 hours post-challenge. All participants will be treated with trimethoprim 160 mg / sulfamethoxazole 800 mg orally twice daily for five days, or ciprofloxacin (500 mg orally twice daily for three days), or, alternatively, ampicillin (500 mg orally four times daily for three days).

Early antibiotic treatment after challenge may commence when any of the following criteria are identified and a study physician considers it to be warranted:

- When volunteers meet the primary endpoint (clinical definition of shigellosis)

OR

- Oral temperature ≥ 39°C

OR

	- Any other reason warranting the early treatment in the physician's opinion If, because of illness, a participant is unable to take oral antibiotics, intravenous antibiotics (e.g., ciprofloxacin) may be given at an appropriate dose based on weight and clinical status. Discharge Criteria All participants will be discharged from the inpatient unit when they are well, have taken at least 2 doses of antibiotics, and have had at least two consecutive stool cultures negative for the challenge strain. Post-Challenge Follow-Up Participants will return for post-challenge assessments at 15, 29, 57, and 90 days post-challenge. A follow-up visit approximately 6 months after challenge will be performed to inquire about the occurrence of any new chronic health conditions (such as reactive arthritis, conjunctivitis, urethritis), serious health events, or hospitalizations, and to complete a functional bowel survey to assess for new-onset chronic bowel problems.
Study Procedures	Screening and Consenting Initial screening will occur under a separately approved general screening protocol over the course of 1 – 2 visits. Baseline laboratories will be drawn, including sera for the analysis of the *S. flexneri*- and *S. sonnei*-IgG levels within 90 days of enrollment. Only participants who have low titers to the challenge strains, as determined by O-antigen-specific antibodies in the serum, will be eligible for enrollment. Eligible and willing participants will be invited to participate in an informed consent process where study information is presented in writing (ICF), view an audio/visual study brief, complete a comprehension assessment, and consent to study participation by signing the informed consent document. Inpatient Admission and Challenge Each group will be admitted to the inpatient unit on Day -2 or -1. The day after admission, Day 1, each participant will be challenged with approximately 1500 cfu of the fully virulent *Shigella* strain after a ≥90-minute fast and immediately following ingestion of a bicarbonate solution. Volunteers will fast for ≥90 additional minutes after challenge. Participants will be selected for the rechallenge randomly, if they are eligible and willing to continue with the study. The duration of the active study period for participants who receive one dose of challenge is approximately 6 months; for participants who receive both challenges, the active study period is about 9 months from enrollment, not including up to 60 days for screening. Follow-Up Phase Safety assessments and blood for immunology will be performed at all study visits. The timeframe for the collection of AEs begins at the time of Investigational Product administration through 28 days after receipt of the challenge strain. Additionally, Adverse Events of Special Interest (AESI) and Serious Adverse Events (SAEs) will be collected for approximately 180 days from last challenge. Outcome Adjudication Committee

	To obtain an unbiased determination of the efficacy outcomes, a blinded independent outcome adjudication committee will evaluate challenge outcome data after completion of the inpatient phase of the study for all cohorts.
Primary Objective	To evaluate the cross-species protection conferred by a re-challenge with a *Shigella* species of a different serotype.
Primary Endpoint	The primary endpoint for outcomes following initial challenge and heterologous re-challenge is shigellosis [1], defined as: - severe diarrhea OR - moderate diarrhea with fever OR with one or more moderate constitutional or enteric symptom OR ≥2 episodes of vomiting in a 24-hour period OR - dysentery: ≥2 loose stools with gross blood (hemoccult positive) in 24 hours AND fever OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours
Secondary Objective	To determine effects of previous challenge when re-challenged with *Shigella* of a different serotype on stool output and clinical symptoms.
Secondary Endpoints	Secondary endpoints for this study following initial challenge and heterologous re-challenge are: - Maximum 24-hour stool output - Percent of participants with severe diarrhea - Percent of participants with diarrhea of any severity - Total weight of grade 3-5 stools per participant - Percent of participants with nausea, vomiting, anorexia, abdominal pain/cramps rated as moderate to severe - Percent of participants who meet the definition of dysentery - Mean/median time to onset of diarrhea - Number of participants with more severe diarrhea (defined as ≥ 10 loose [grade 3-5] stools within 24h or ≥1000 gr loose [grade 3-5] stools within 24h) - Number of participants with fever - *Shigella* clinical severity score post-challenge - Number of cfu of the challenge strain per gram of stool Note: Period of data collection for all secondary endpoints is during the inpatient period.
Exploratory Objectives	1. To determine IgG and IgA responses to *Shigella* species specific lipopolysaccharide (LPS) upon challenge or rechallenge. 2. To utilize novel analyses of immune responses, including antigen arrays, to identify potentially protective immune responses in individuals protected from shigellosis upon challenge or rechallenge. 3. To identify potential protective antigens on the challenge organisms. 4. To confirm that the serum IgG to the O-antigen is a correlate of protection for shigellosis. 5. To assist in the development of international standards against *Shigella* antigens. 6. To measure mucosal and systemic immune responses to experimental infection.

	7. To obtain and archive samples for future proteomics, inflammatory marker, microbiome, and/or transcriptomics and systems biology efforts based on the recently published consensus schedule and events table [2]. 8. To evaluate the cognitive and sleep impact of acute diarrhea using psychomotor vigilance testing (PVT) and actigraphy. 9. To evaluate serum bactericidal antibody (SBA) titers against *S. flexneri* 2a 2457T and *S. sonnei* 53G (responders defined as ≥4-fold increase in SBA titer at designated timepoints post-initial challenge and heterologous re-challenge. 10. To evaluate the ability of wearable-collected data to predict clinical and/or microbiological endpoints.
Definitions	Stool will be graded based on a standard stool grading scale as follows [3]: Grade 1 = Fully formed (normal) Grade 2 = Soft (normal) Grade 3 = Thick liquid (diarrheal) Grade 4 = Opaque watery (diarrheal) Grade 5 = Rice-water (diarrheal) • More severe diarrhea: ≥10 loose (grade 3-5) stools within 24 hours (h) or ≥1000 grams loose (grade 3-5) stools within any 24-hour window • Severe diarrhea: ≥6 loose (grade 3-5) stools within 24h or >800 grams loose (grade 3-5) stools within any 24-hour window • Moderate diarrhea: 4 to 5 loose (grade 3-5) stools within 24h or 401-800 grams loose (grade 3-5) stools within any 24-hour window • Mild diarrhea: ≥2 loose (grade 3-5) stools weighing ≥200 grams within any 48-hour window or 1 loose stool weighing ≥300 grams not meeting the definition for moderate or severe • Fever: measured oral temperature ≥ 38°C confirmed within about 20 minutes • Dysentery: ≥2 loose stools (grade 3-5) with gross blood (hemoccult positive) in 24 hours AND fever OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours • Constitutional/enteric symptom: nausea, abdominal cramps/pain, myalgia, arthralgia, malaise • Shigellosis disease severity will be assessed as previously described [4].
Inclusion Criteria	1. Healthy adults, male or female, aged 18 to 50 years (all inclusive) at the time of enrollment. 2. General good health, without clinically significant medical history, physical examination findings, or clinical laboratory abnormalities per judgment of PI. 3. Willingness to participate in the study after all aspects of the protocol have been explained and written informed consent obtained. 4. Completion of a training session and demonstrated comprehension of the protocol procedures and knowledge of *Shigella*-associated illness by passing a written examination (70% passing score). 5. Availability for the study duration, including all planned follow-up visits. 6. Female participants must have a negative pregnancy test at screening and prior to each challenge. **7.** Female participants must agree to avoid pregnancy for 29 days following the last challenge dose by use of an efficacious hormonal or barrier method of birth control during the study. Abstinence is acceptable. Female participants unable to bear children must have this documented (e.g., tubal ligation or hysterectomy).

	8. Willingness to refrain from participation in a study of another investigational agent for 90 days following the last challenge dose.
Exclusion Criteria	1. Presence of a significant medical or psychiatric condition that in the opinion of the investigator precludes participation in the study. Some medical conditions that are adequately treated and stable would not preclude entry into the study. 2. Clinically significant abnormalities in screening on physical exam or screening laboratory results as determined by PI or PI in consultation with the research monitor and Sponsor. 3. Recent receipt of another investigational product (within 30 days before enrollment). 4. Positive enzyme-linked immunosorbent assay (ELISA) and confirmatory tests for human immunodeficiency virus (HIV). 5. Positive hepatitis C (HCV) ELISA and confirmatory test (e.g., HCV ribonucleic acid (RNA)). 6. Positive hepatitis B virus surface antigen (HBsAg) by ELISA. 7. Use of any medication that affects immune function (e.g., corticosteroids and others) within 30 days preceding the first challenge or planned use during the active study period (topical and ophthalmologic steroids are allowable). 8. Evidence of impaired immune function or immune compromise (known immunodeficiency syndrome; either congenital, acquired, or iatrogenic; active autoimmune disease; repeated serious infections without known cause). 9. IgA deficiency (serum IgA < 7 mg/dL or below the limit of detection of the assay). 10. Positive blood test for HLA-B27. 11. Personal or family history of an inflammatory arthritis. 12. Currently pregnant or nursing. 13. Have household contacts who are < 2 years old or > 80 years old or infirm or immunocompromised 14. Employment as a health care worker with direct patient care, in a daycare center (for children or the elderly), or direct food handler; includes individuals who work directly with food in commercial establishments 15. Evidence of current alcohol or drug dependence, or history of dependence in the last 6 months. 16. Recent vaccination (including licensed vaccines) or receipt of an investigational product (within 30 days before challenge through 90 days following the last challenge dose). Annual influenza vaccine, an emergency authorized or licensed COVID-19 vaccine, or a TdaP or Td booster may be administered beyond 14 days before and after each challenge. 17. Treatment with immunoglobulins or blood products within 3 months of challenge. 18. Current or prior history of inflammatory bowel disease or irritable bowel syndrome or abnormal stool pattern (>3/day or <3/week, or loose or liquid stools). 19. Chronic use of anti-diarrheal, anti-constipation, or antacid therapy; or use of these medications in the 7 days prior to challenge. 20. Use of proton pump inhibitors or H2 blockers (H2-receptor antagonists) within 48 hours prior to challenge. 21. Use of antibiotics within 7 days prior to challenge. 22. Known allergy to any 2 of the following antibiotics: ciprofloxacin, trimethoprim-sulfamethoxazole, or a penicillin 23. Symptoms of travelers' diarrhea (TD) associated with travel to countries where *Shigella* or other enteric infections are endemic (most of the developing world) within 3 years prior to challenge OR planned travel to endemic countries during the active study period.

	24. History of shigellosis, *Shigella* vaccination or challenge, or a laboratory worker with known exposure to *Shigella* within the last 5 years. 25. Serum IgG titer > 2500 to either *Shigella flexneri* 2a or *Shigella sonnei* LPS. 26. Any other criteria which, in the investigator's opinion, would compromise the ability of the volunteer to participate in the study, the safety of the study, or the results of the study.

Safety Monitoring	The research monitor and PI will review any safety concerns.
	
	Research Monitor: The research monitor will function as an independent safety advocate for participants. This individual will have the following responsibilities:
	• Evaluate ongoing safety data and make recommendations in order to ensure participants' safety as required
	• Be available for consultation by the clinical investigative team through the period of the clinical study in which there is an interaction with human participants
	• Be available to review all SAEs and other unanticipated problems involving risk to participants
	• Provide clinical advice, in accordance with the study protocol, on the clinical management of participants. This advice may include, but is not limited to:
	  • Decisions on "borderline" eligibility for enrollment
	  • Confirmation and discussion of treatment decisions for difficult clinical situations
	  • Must document all clinical decisions including date, time, and signature
	  • Must communicate all decisions to the study PI and other study investigators, which must be stored with participant source documents
	
	All SAEs must be reported, whether or not regarded as possibly attributable to the test articles or antibiotic. SAE reports will be provided to the Sponsor, research monitor, and IRB(s). The investigator must report SAEs within one calendar day of becoming aware of the event by telephone, fax, or e-mail (if appropriate) to the study contact for reporting SAEs as described in the protocol.

Study Duration	The expected duration of the trial is approximately 24 months. Duration of study participation is approximately 11 months per volunteer from first contact, screening, to completion of the last outpatient follow-up visit, 180 days post last challenge. Participants will complete 1 – 2 screening visits, a consenting visit, 1 or 2 inpatient stays of approximately 10 days each, and 5 (if only 1 challenge) or 8 (if 2 challenges) post-challenge outpatient follow-up visits. Screening and consenting will occur in the 60 days prior to enrollment.
Sample Size	A total of up to 54 participants will be challenged in this study (up to 110 will be consented to ensure a full complement of volunteers). Based on Table S1, participants are delineated as follows: • *S. flexneri* 2a 2457T single challenge (n=16; 8 (2 in Group 2A1 and 6 in Group 2A2) not selected for rechallenge, 8 rechallenge controls in Group 1B1) Rechallenge controls are naïve subjects enrolled alongside veteran subjects (receiving their second challenge) and both receive the same challenge agent to allow a fair comparison for the attack rate across the group.

	• *S. sonnei* 53G single challenge (n=up to 8; 3 (2 in Group 1A1 and 1 in Group 1A2) not selected for rechallenge, 5 rechallenge controls in Group 2B1) • *S. flexneri* 2a 2457T challenge followed by *S. sonnei* 53G challenge (n=11 veterans in Group 2B1) • *S. sonnei* 53G challenge followed by *S. flexneri* 2a 2457T challenge (n=19 veterans in Groups 1B1 and 1B2) Presuming a shigellosis rate of 70% in naïve participants, a sample size of 16 participants will yield 95% confidence intervals (asymptotic estimates) of 48-92%, while a sample size of 8 participants will yield 95% confidence interval (asymptotic estimates) of 38-100%. Presuming an ability to pool naïve participants, a sample size of 19 participants in the previously *S. sonnei* 53G challenged arm and 16 participants in the *S. flexneri* 2457T naïve arm provides a >80% power to detect a shigellosis risk difference of 46% presuming a ≥70% attack rate in *Shigella* naïve participants, while a sample size of 11 participants in the previously *S. flexneri* 2457T challenged arm and 8 participants in the *S. sonnei* 53G naïve arm provides a >80% power to detect a shigellosis risk difference of 60% presuming a ≥70% attack rate in *Shigella* naïve participants (based on a Pearson's chi-squared test for proportional differences and a 2-sided *alpha*=0.05).
Statistical methods and analysis	All AEs will be summarized and compared between groups. Safety data, including AEs, stool information, specified vital signs, and laboratory tests, will be listed by study subject. Descriptive statistics (n, mean, standard deviation, median and ranges for continuous variables, percentages for categorical variables) will be compiled for each group. The attack rate of shigellosis will be estimated along with 95% confidence intervals (both asymptotic estimates and exact) for each study group. Attack rates between *Shigella* naïve participants and previously challenged participants will be compared using a Pearson's chi-squared or Fisher's exact test, as appropriate. Serologic and cellular assessments of the immune response will be conducted to assess for potentially protective responses in the volunteers resistant to the development of shigellosis. Comparisons across groups of single immune parameters reported as continuous variables (e.g., reciprocal $\log_{10}$ titers) will be made utilizing t-tests and one-way analysis of variance, Wilcoxon rank-sum, or Kruskal-Wallis H tests, as appropriate. Comparisons of responses within groups over time will be made utilizing appropriate paired analyses. Immune parameters that are nominal in nature (e.g., responder status) will be compared using chi-squared or exact tests, as appropriate. Additionally, comparisons of immune response profiles, assessing multiple immune parameters simultaneously, will be explored utilizing multivariable regression models, principal component analyses, and/or other multivariable methods, as appropriate. All hypothesis testing will be conducted using a 2-sided *alpha*=0.05.

IV. BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

1. Background Information-Clinical Significance and Pathogenicity of *Shigella*

Shigella, a major cause of bacillary dysentery, is an enteric bacterium that can cause inflammatory diarrhea. *Shigella* has long been recognized as a cause of moderate and severe diarrhea and dysentery, with a high incidence among children residing in low- and middle-income countries [5, 6]. Recent studies highlight the continued burden of disease and provide data for burden estimates [7-11]. In addition to an estimated 212,438 annual deaths in people of all ages [12], children under 5 years of age experience nearly 75 million cases of shigellosis each year, leading to stunting and wasting [12-14]. *Shigella* also poses a significant enteric disease threat to deploying military forces and international travelers [15].

2. Rationale for Current Study

This is a human challenge study designed to assess the cross-protective efficacy and markers of protection after challenge and heterologous rechallenge with different *Shigella* strains in naïve volunteers. The study would look for novel, cross-protective *Shigella* antigens which could improve upon the current requirement for serotype-specific O-antigens from the most common species, *S. flexneri* 2a, 3a, and 6, and *S. sonnei*. Such antigens could be used as carrier proteins in third-generation glycoconjugate vaccines or act as stand-alone subunit vaccines to help broaden vaccine coverage.

3. Previous Experience

The *Shigella* Controlled Human Infection Model (CHIM) has been in use to evaluate the efficacy of investigational *Shigella* vaccines since the studies of Shaughnessy and colleagues in 1946 among prison inmates in Joliet, Illinois [16]. Since then, CHIM trials evaluating *Shigella* vaccine candidates have been conducted at several sites in the United States, mostly with *S. flexneri* 2a (strain 2457T) [17-19] and *S. sonnei* (strain 53G) [20, 21], but also with wild-type and toxin-minus mutants of *S. dysenteriae* type 1 [19, 20, 22-27]. Additionally, trials with 53G have been conducted in Thailand [28, 29]. Previous field and CHIMs studies have indicated that infection with specific *Shigella* serotypes will induce protection against of a similar "O" serotype but the antigens associated with cross-serotype protection are not as well understood (Levine MM et al. 2007).

S. flexneri 2a Strain 2457T

S. flexneri 2a has had the greatest range of doses tested for strain 2457T; ranging from 100 to 1 x 10^8 colony forming units (cfu) [19].

Experimental *S. flexneri* 2a challenge was first attempted in the late 1960s in an effort to develop a safe and reproducible model for rapid and accurate evaluation of the efficacy of candidate *Shigella* vaccines in humans [13]. The 2457T *S. flexneri* 2a strain that will be utilized in this study as a challenge strain is a well-characterized *Shigella* strain manufactured under current Good Manufacturing Practice (cGMP) conditions at the WRAIR Pilot BioProduction Facility in Silver Spring, Maryland. This strain was originally isolated from a clinically ill patient in Japan in the early 1950s and has most frequently been given to volunteers participating in IND studies evaluating treatment or preventive intervention for the control and clinical management of shigellosis [19]. The 2457T strain is also covered by its own manufacturing master file registered with the FDA (BB-MF-3408). In all, more than 460 people have been challenged with the strain in the published literature, and of the *Shigella* strains used in challenge studies, this is the least likely to cause dysentery. The 2457T strain has been used in 15 published trials at doses ranging from 100 to 10^8 cfu [19], and additional challenge studies carried out by the Center for

Immunization Research (CIR) in recent years that have yet to be published. Diarrhea attack rates are somewhat dose-related under 1400 cfu, but plateau at higher doses to approximately 60 to 80% [19]. The dose of 1500 cfu was confirmed in multiple studies at the University of Maryland and the CIR [23, 30, 31]. In total, the CIR has conducted 5 inpatient challenge studies with the 2457T strain since 2001, with the most recent being in 2015. 1500 cfu is now the standard dose in challenge studies. This strain is susceptible to the antibiotics that will be used for treatment (ciprofloxacin, trimethoprim-sulfamethoxazole, ampicillin).

S. sonnei Strain 53G

The second most commonly studied strain (*S. sonnei* 53G) has historically been used in low inoculum doses, ranging between 400 and 500 organisms, with relatively low diarrhea and dysentery attack rates. This strain has been utilized at a number of sites in the US (in 296 adults) and in Thailand (in 56 adults), including at the CIR by Kirkpatrick and colleagues (Unpublished). Recently, a lyophilized preparation of this strain has been manufactured under cGMP conditions at the WRAIR Pilot BioProduction Facility (PBF) to simplify the inoculum preparation process. A dose-ranging study was conducted at Cincinnati Children's Hospital under an IND for the challenge strain to better characterize and evaluate this challenge strain. The challenge model demonstrated an acceptable safety profile, with no signs of early onset or particularly aggressive disease. Among subjects receiving the highest dose (1760 cfu), 70% developed moderate to severe diarrhea, 50% had dysentery, and 40% had fever. A dose in the range of 1500 to 2000 cfu was identified as the optimal dose for a reproducible diarrhea attack rate of ≥60% [32]. This strain is susceptible to the antibiotics that will be used for treatment (ciprofloxacin, trimethoprim-sulfamethoxazole, ampicillin).

4. Dose and Administration Rationale

Multiple studies have been carried out over the last 40 years to refine the challenge models and improve reproducibility. Most recently, a convening of the experts from the sites that conduct *Shigella* challenge studies was hosted by the Bill and Melinda Gates Foundation, resulting in a series of consensus guidelines on the conduct of these studies [1, 3]. This study is in compliance with those guidelines.

V. STUDY HYPOTHESIS

Challenge with one species of *Shigella* (*Shigella flexneri* 2a or *Shigella sonnei*) will induce some cross-protective immunity such that a subset of volunteers will be protected upon challenge with the second strain.

VI. OBJECTIVES

1. Primary Objective:

To evaluate the cross-species protection conferred by a re-challenge with a *Shigella* species of a different serotype.

2. Secondary Objective:

To determine effects of previous challenge when re-challenged with *Shigella* of a different serotype on stool output and clinical symptoms.

3. Exploratory Objectives:

1. To determine IgG and IgA responses to *Shigella* species-specific lipopolysaccharide (LPS) upon challenge or rechallenge.

2. To utilize novel analyses of immune responses, including antigen arrays, to identify protective immune responses in individuals protected from shigellosis upon challenge or rechallenge.
3. To identify potential protective antigens on the challenge organisms.
4. To confirm that the serum IgG to the O-antigen is a correlate of protection for shigellosis.
5. To assist in the development of international standards against *Shigella* antigens.
6. To measure mucosal and systemic immune responses to experimental infection.
7. To obtain and archive samples for future proteomics, inflammatory marker, microbiome, and/or transcriptomics and systems biology efforts based on the recently published consensus schedule and events table [2].
8. To evaluate the cognitive and sleep impact of acute diarrhea using psychomotor vigilance testing (PVT) and actigraphy.
9. To evaluate serum bactericidal antibody (SBA) titers against *S. flexneri* 2a 2457T and *S. sonnei* 53G (responders defined as ≥4-fold increase in SBA titer at designated timepoints post-initial challenge and heterologous re-challenge.
10. To evaluate the ability of wearable-collected data to predict clinical and/or microbiological endpoints.

VII. ENDPOINTS

1. Primary Endpoint

The primary endpoint for outcomes following initial challenge and heterologous re-challenge is the onset of shigellosis, defined as:

- Severe diarrhea: ≥6 loose (grade 3-5) stools within 24h or >800gr loose (grade 3-5) stools within 24h

  OR

- Moderate diarrhea (4 to 5 loose stools within 24 hr or 401-800gr loose (grade 3-5) stools within 24h) with fever OR with one or more moderate constitutional or enteric symptom OR ≥2 episodes of vomiting in a 24-hour period

  OR

- Dysentery: ≥2 loose stools with gross blood (hemoccult positive) in 24 hours **AND** fever **OR** ≥1 moderate constitutional/enteric symptom **OR** ≥2 episodes of vomiting in 24 hours

Fever: oral temperature ≥ 38°C confirmed within about 20 min

Constitutional/Enteric Symptom: nausea, abdominal cramps/pain, myalgia, arthralgia, malaise

2. Secondary Endpoints

Secondary endpoints for this study following initial challenge and heterologous re-challenge are:

- Maximum 24-hour stool output
- Percent of participants with severe diarrhea
- Percent of participants with diarrhea of any severity
- Total weight of grade 3 – 5 stools per participant
- Percent of participants with nausea, vomiting, anorexia, abdominal pain/cramps rated as moderate to severe
- Percent of participants who meet the definition of dysentery

- Mean/median time to onset of diarrhea
- Number of participants with more severe diarrhea (defined as ≥10 loose [grade 3-5] stools within 24h or ≥1000 gr loose [grade 3-5] stools within 24h)
- Number of participants with fever
- *Shigella* clinical severity score post-challenge
- Number of cfu of the challenge strain per gram of stool

Note: Period of data collection for all secondary endpoints is during the inpatient period.

VIII. STUDY DESIGN

1. Overview

This study will be conducted in the isolation unit at the CIR Inpatient Unit, located at the Johns Hopkins Bayview Medical Center. This study will be a single-center human challenge study designed to assess the cross-protective efficacy and markers of protection after challenge and heterologous rechallenge with different *Shigella* strains in naïve healthy adult volunteers (see Figure 1: Study Schema).

Description of study groups as shown in table 1 below:

Cohort 1, group 1A1: 13 participants will be challenged with 1500 cfu of *S. sonnei* 53G. At least 3 months later, group 1B1: 11 of the participants from group 1A1 (who are willing and eligible to proceed to another challenge), along with up to 8 naïve participants (group 1B1), will be challenged with 1500 cfu of *S. flexneri* 2a. Up to 9 newly recruited naïve participants will be challenged with 1500 cfu of *S. sonnei* 53G (Group 1A2) and later 8 of these volunteers willing and eligible for rechallenge will be rechallenged with 1500 cfu of *S. flexneri* 2a (group 1B2).

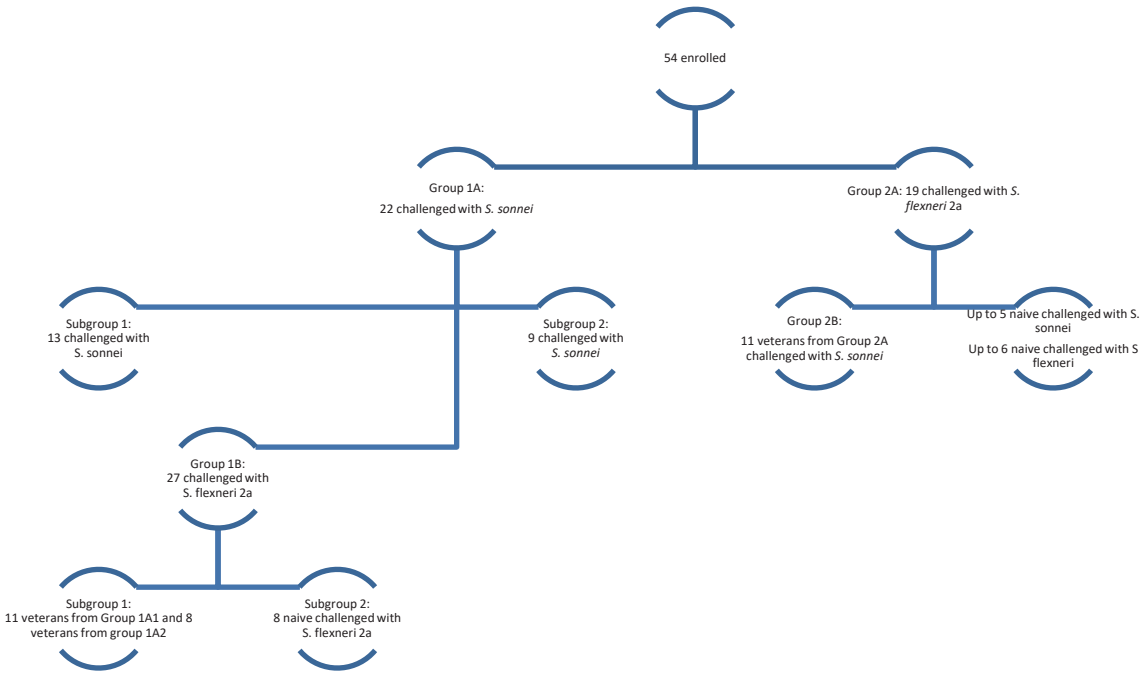
Cohort 2, group 2A1: 13 naive participants will be challenged with 1500 cfu of *S. flexneri* 2a. At least 3 months later, group 2B1: up to 11 of those volunteers from Group 2A1 (who are willing and eligible to proceed to another challenge), along with 5 or more naïve participants (group 2B1), will be challenged with 1500 cfu of *S. sonnei* 53G, and another group of up to 6 naive participants will be challenged with 1500 cfu of *S. flexneri* 2a (group 2A2). Each volunteer will be followed for 6 months from the time of their last challenge.

Table 1: Description of Study Groups

Group/ cohort	N	Sub-group	N naïve	N veterans	Challenge strain (1500 cfu/dose)
1A	13	1	13	0	*S. sonnei* 53G
	9	2	9	0	*S. sonnei* 53G
1B	19	1	Up to 8	11 from 1A1	*S. flexneri* 2a 2457T
	8	2	0	8 from 1A2	*S. flexneri* 2a 2457T
2A	13	1	13	0	*S. flexneri* 2a 2457T
	Up to 6	2	Up to 6	0	*S. flexneri 2a 2457T*
2B	Up to 16	1	5	Up to 11 from 2A1	*S. sonnei* 53G

Breakdown of study cohorts/groups: Each cohort is defined by which challenge agent is administered first (e.g., Cohort 1, *S. sonnei*, Cohort 2, *S. flexneri* 2a). The groups refer to the admission; Group A is the first admission/challenge per cohort, B, the second admission/rechallenge. Within the A groups, all volunteers are naïve; within each of the B groups, up to 20 volunteers will be veterans from the A group and up to 10 volunteers will be naïve. The numbers after the letter e.g. 1A1, denote subgroups that occurred at different admissions.

Figure 1: Study Schema



The CIR Inpatient Unit is a 30-bed dorm-like facility for clinical trials. Each group will be admitted together to the inpatient unit for up to 10 days or longer. The day after admission, each participant will be challenged with approximately 1500 cfu of the fully virulent *Shigella* strain indicated after a 90-minute fast and immediately following ingestion of a bicarbonate solution. Volunteers will fast for 90 additional minutes after challenge.

The duration of the active study period for participants who receive one dose of challenge is approximately 6 months; for participants who receive both challenges, the active study period is about 9 months from enrollment, not including up to 60 days during the screening period.

2. Description of Study Design

This is a *Shigella* controlled human infection study with a goal to assess the cross-protective efficacy and markers of protection after challenge and heterologous rechallenge with different *Shigella* strains in naïve healthy, 18 to 50 year-old, non-pregnant volunteers.

The study will be divided into 3 parts; about 2/3 of the participants will repeat parts 2 and 3:

1. Screening (usually 2 visits; some visits will be performed under a separate, IRB-approved screening protocol) (see Section XI for details)
2. Admission and challenge phase
3. Follow-up phase (outpatient visits at Study Days 15, 29, 57, 90, and 180)

All potential volunteers will be screened for titers to *S. flexneri* and *S. sonnei* IgG. Those who meet eligibility criteria will be admitted to the inpatient unit and receive the challenge strain (Investigational Product). Blinding will not be employed in this study as all subjects in each cohort will be receiving the same challenge strain. However, the adjudication of clinical outcomes will be blinded to both strain and cohort. Participants are considered enrolled once they receive the investigational challenge. Volunteers will be monitored by clinical staff 24/7 while on the inpatient unit. They will be assessed for AEs and symptoms of *Shigella* illness daily post-challenge. All stools will be collected and assessed for grade, weight, and blood daily post-challenge until participants are eligible for discharge. Stool samples for culture and research

testing will be collected daily; blood, nasal swabs, and saliva will be collected at specified time points as per the schedule of events. Volunteers will be eligible for discharge once they have received at least 2 doses of antibiotics, clinical symptoms are resolved or resolving, and they have at least 2 consecutive negative stools for *Shigella*.

Trial Schedule:
See Table 9: Schedule of Procedures for Subjects Receiving One Challenge and Table 10: Schedule of Procedures for Subjects Receiving Two Challenges.

IX. STUDY POPULATION

1. Selection and Enrollment of Participants

Participants will be recruited from the Baltimore, MD-Washington, DC region and surrounding areas via advertisements and word of mouth and screened at the CIR. A sufficient number will be screened to provide for the number planned to challenge, with sufficient alternates to allow for last-minute dropouts. The total target is 65 participants enrolled. Once challenged, subjects will not be replaced.

2. Inclusion Criteria:

Participants must meet **all** criteria listed below to be included in the study:

1. Healthy adults, male or female, aged 18 to 50 years (all inclusive) at the time of enrollment.
2. General good health, without clinically significant medical history, physical examination findings, or clinical laboratory abnormalities per judgment of PI.
3. Willingness to participate in the study after all aspects of the protocol have been explained and written informed consent obtained.
4. Completion of a training session and demonstrated comprehension of the protocol procedures and knowledge of *Shigella*-associated illness by passing a written examination (70% passing score).
5. Availability for the study duration, including all planned follow-up visits.
6. Female participants must have a negative pregnancy test at screening and prior to each challenge.
7. Female participants must agree to avoid pregnancy for 29 days following the last challenge dose by use of an efficacious hormonal or barrier method of birth control during the study. Abstinence is acceptable. Female participants unable to bear children must have this documented (e.g., tubal ligation or hysterectomy).
8. Willingness to refrain from participation in a study of another investigational agent for 90 days following the last challenge dose.

3. Exclusion Criteria:

Participants meeting **any** of the exclusion criteria listed below must be excluded from participating in the trial:

1. Presence of a significant medical or psychiatric condition that in the opinion of the investigator precludes participation in the study. Some medical conditions that are adequately treated and stable would not preclude entry into the study.
2. Clinically significant abnormalities in screening on physical exam or screening laboratory results as determined by PI or PI in consultation with the research monitor and Sponsor.
3. Recent receipt of another investigational product (within 30 days before enrollment).
4. Positive enzyme-linked immunosorbent assay (ELISA) and confirmatory tests for human immunodeficiency virus (HIV).
5. Positive hepatitis C Virus (HCV) ELISA and confirmatory test (e.g., HCV RNA).
6. Positive hepatitis B virus surface antigen (HBsAg) by ELISA.

7. Use of any medication that affects immune function (e.g., corticosteroids and others) within 30 days preceding the first challenge or planned use during the active study period (topical and ophthalmologic steroids are allowable).
8. Evidence of impaired immune function or immune compromise (known immunodeficiency syndrome; either congenital, acquired, or iatrogenic; active autoimmune disease; repeated serious infections without known cause).
9. IgA deficiency (serum IgA < 7 mg/dL or below the limit of detection of the assay).
10. Positive blood test for HLA-B27.
11. Personal or family history of an inflammatory arthritis.
12. Currently pregnant or nursing.
13. Have household contacts who are < 2 years old or > 80 years old or infirm or immunocompromised.
14. Employment as a health care worker with direct patient care, in a daycare center (for children or the elderly), or direct food handler; includes individuals who work directly with food in commercial establishments
15. Evidence of current alcohol or drug dependence, or history of dependence in the last 6 months.
16. Recent vaccination (including licensed vaccines) or receipt of an investigational product (within 30 days before challenge through 90 days following the last challenge dose). Annual influenza vaccine, an emergency authorized or licensed COVID-19 vaccine, or a TdaP or Td booster may be administered beyond 14 days before and after each challenge.
17. Treatment with immunoglobulins or blood products within 3 months of challenge.
18. Current or prior history of inflammatory bowel disease or irritable bowel syndrome or abnormal stool pattern (>3/day or <3/week, or loose or liquid stools).
19. Chronic use of anti-diarrheal, anti-constipation, or antacid therapy; or use of these medications in the 7 days prior to challenge.
20. Use of proton pump inhibitors or H2 blockers (H2-receptor antagonists) within 48 hours prior to challenge.
21. Use of antibiotics within 7 days prior to challenge.
22. Known allergy to any 2 of the following antibiotics: ciprofloxacin, trimethoprim-sulfamethoxazole, or a penicillin.
23. Symptoms of travelers' diarrhea (TD) associated with travel to countries where *Shigella* or other enteric infections are endemic (most of the developing world) within 3 years prior to challenge OR planned travel to endemic countries during the active study period.
24. History of shigellosis, *Shigella* vaccination or challenge, or a laboratory worker with known exposure to *Shigella* within the last 5 years.
25. Serum IgG titer > 2500 to either *Shigella flexneri* 2a or *Shigella sonnei* LPS.
26. Any other criteria which, in the investigator's opinion, would compromise the ability of the volunteer to participate in the study, the safety of the study, or the results of the study.

4. Continuing Eligibility Criteria to Proceed to Cohort 1B or 2B

1. Must continue to meet inclusion criteria above.
2. Must not meet any of exclusion criteria 1-21 and 24 (IgA, HLA-B27, HIV, HCV, and HBsAg will not be repeated).
3. All study subjects with Serious Adverse Events (SAEs) related to the primary challenge will be excluded from a repeat (second) challenge.

5. Access to Medical Records

A medical history will be obtained directly from each participant. Medical records will not be requested unless there is a need to clarify a question in the participant's medical history or if the participant had an intercurrent illness or injury requiring medical care during the study. Medical records will not be requested without a signed medical release and the informed consent of the

participant. Informed consent for the medical release will be obtained from each participant at the beginning of the study.

X. STUDY PROCEDURES

1. Screening

The CIR will use a screening protocol approved by the Johns Hopkins School of Public Health (JHSPH) Institutional Review Board (IRB) in recruiting participants for this study. The screening protocol is entitled "Screening of adult volunteers for eligibility to participate in clinical studies evaluating investigational vaccines, antimicrobial agents, or disease prevention measures or the pathogenesis of infectious agents" CIR 200, JHSPH IRB 00010083. Participants will be made aware that the screening process may take several visits to complete. Once consented, using this screening protocol, a medical history/exam and a series of clinical laboratory tests will be completed to rule out occult illness and pregnancy (as per Section XI, 8). Participants who have ≤ 2 mild (grade 1) non-hematologic abnormalities may be included if the PI determines that their participation will not present undue risk to the participant. Participants with > 2 mild abnormalities may be included in the study at the discretion of the PI with concurrence from the research monitor. Participants with clinical laboratory abnormalities >grade 1 will not participate in this clinical trial.

To ensure comprehension of the study, all participants will have to pass a written examination before inclusion in the study. Participants who meet all inclusion criteria and none of the exclusion criteria, pass the comprehension test, and sign the study Informed Consent Form (ICF) may be eligible for the study. Informed consent is an ongoing process which includes the ICF. Participants will watch an audio/visual presentation of the study after which they will be given an opportunity to ask any questions. Each prospective participant will be given the written, IRB-approved informed consent, allowed ample time to read the consent, allowed to ask questions about the study, have his/her questions answered, and given time to decide if he/she would like to participate in the study. To document participants' understanding of informed consent, immediately before the consent is signed, the person obtaining consent will administer a comprehension test. Incorrect answers will be discussed with participants to reinforce the consent. A final acceptable test score is 70% or more answered correctly. Participants who fail the comprehension test on the first attempt may retake the comprehension test on the same day, or they may come back on a separate visit to retake the test. Participants failing after two attempts are not eligible for study enrollment. No coercion or influence is allowed in obtaining participants' consent. Before participants participate in the study, consent forms will be signed and dated by participants as well as by the PI or designee. Participants will receive copies of the signed consent prior to participation. As part of the consent process, participants will also be asked to read and sign additional IRB-approved forms including but not limited to, a Medical Records/Lab Results Release, alternate information form, and inpatient guidelines, with an opportunity to ask questions, if relevant.

As part of the pre-inpatient period, eligible subjects may be asked to perform a 5-minute baseline psychomotor vigilance test (PVT). Additionally, subjects will be provided a smartphone and actigraphy devices to be worn starting up to 21 days prior to challenge through the first post-discharge follow-up visit (study day 15 ± 2 days).

2. Clinical Evaluations

i. Monitoring During Inpatient Phase

Eligible participants will be admitted to the CIR inpatient facility on the Johns Hopkins Bayview campus on Day -2 or -1. Once challenged (Day 1), they will be monitored daily while in the inpatient unit for the development of shigellosis, including general,

gastrointestinal, and systemic signs and symptoms, have medical conditions reviewed, and adverse effects recorded. This will include examination by a study physician/nurse practitioner/physician assistant and solicitation of adverse events (as per list in Section XIII, 5). Additionally, participants will be examined for symptoms and signs of dehydration, including thirst, dizziness on standing, decreased skin turgor, and dryness of mucous membranes. Vital signs will be recorded three times daily (four times on challenge day), and more often when participants are ill. If participants develop moderate or severe diarrhea, postural blood pressure and pulse will be measured as necessary for clinical management according to the judgment of the clinician.

A clinician will conduct a daily medical interview and focused physical exam to assess health status and treat as indicated. All stools will be collected for weighing and grading [3]. Following *Shigella* challenge, up to 3 stool samples will be collected daily for culture as per study specific procedure (SSP) starting the day after challenge. If a participant is unable to provide a stool sample by 1300 hours, s/he will be asked to obtain up to 3 rectal swabs. Swabs will be used starting the day after challenge.

ii. Wearable Devices and PVT Monitoring

An exploratory assessment of the bidirectional relationships between sleep, cognitive performance, and shigellosis will be conducted with the use of continuous wrist actigraphy and PVT monitoring. Heart rate and skin temperature may also be collected by the wearable devices. These outcomes will not be utilized as part of the regulatory safety, immunogenicity, or efficacy evaluations of the study products; they are exploratory in nature and will not be retained in the regulatory file. Missed PVTs will not be considered protocol deviations. Management of symptoms associated with *Shigella* challenge or other illness will have priority over completion of PVTs.

During screening, participants will be issued two wrist-worn devices and a smartphone for collection of actigraphy, heart rate, and skin temperature data. (Each device will collect different information. There will be no GPS information gathered by the devices). Subjects will wear these devices starting up to 21 days prior to challenge through the first post-discharge follow-up visit (study day 15 ± 2 days). Subjects will be asked to complete a brief survey about how they are feeling each day using the smartphone. These surveys will not be used to determine medical history or clinical endpoints; they are exploratory in nature and will not be retained in the regulatory file. Five-minute PVT tests will be performed by each subject up to three times a day. Analyses will explore symptom presence/severity, while adjusting for sleep cycles and other confounding variables, as appropriate. Additional exploratory assessments will include evaluations of immune activation and cognitive performance by use of systems biology parameters. These data will be collected for additional exploratory assessments beyond the scope of this protocol.

iii. Rehydration Procedures

Participants passing grade 3-5 stools post-challenge will be offered Oral Rehydration Solution (ORS) or Gatorade to prevent dehydration, at the same volume as their stool output. For documentation purposes of concomitant medications, ORS will not be considered a concomitant medication; however, intravenous (IV) fluids will.

A participant may be administered IV fluids (clinician discretion) for the following reasons:

- Participant experiences abrupt onset of diarrhea defined by passage of an initial loose/liquid stool of > 300 grams or passage of > 400 grams of loose/liquid stools over 2 hours.
- Participant becomes hypovolemic.

- It is determined necessary by the study physician due to diarrhea with nausea/vomiting and unable to drink enough to keep up with output, or other reason.

Hypovolemia is a significant decrease in blood volume, characterized by either:

- Orthostatic hypotension, confirmed systolic blood pressure (BP) < 90 mmHg and associated symptoms.
- Significant lightheadedness on standing with a confirmed postural change in BP or pulse. Postural vital signs will be measured laying and 2 minutes after standing. A significant change will be any of the following: decrease in systolic BP of > 20 mmHg or diastolic BP of > 10 mmHg, or increase in pulse of > 30 beats/min.

iv. Treatment for Vomiting

Treatment for severe nausea or vomiting may be needed. Participants who experience severe nausea or vomiting may be given oral or intravenous (IV) ondansetron (Zofran).

v. Treatment for Fever or Pain

Acetaminophen, ibuprofen, or naproxen may be used to treat fever or pain.

vi. Other Treatments

In addition to above medications other medications may be given to participants as needed for symptom control (e.g., heartburn), or if a medical problem arises (allergic reaction, insomnia). The most commonly utilized medication, including the indication are as follows:

Medication	**Dosage**	**Indication**	
Ondansetron oral dissolving tablet	4 mg, 8 mg once, can be repeated if not better in 30 minutes	Nausea/vomiting	
Ondansetron IV	0.15 mg/kg IV x 1	Vomiting	Max 16 mg/dose
Acetaminophen	500- 1000 mg q 4-6 hours	Pain, fever	Max 4000 mg/day
Ibuprofen	400-800 mg q 6-8 hours	Pain, fever	Max 2400 mg/day
Naproxen sodium	220-440 mg q 12 hours	Pain, fever	Max 660 mg/day
Naproxen	250-500 mg bid	Pain, fever	Max 1000 mg/day
Benadryl	25-50 mg oral q 6 hrs prn	Itching, insomnia	
Maalox	As directed	Heartburn, indigestion	
Tums	As directed	Heartburn, indigestion	

3. Routine Discharge

Routine discharge is scheduled for approximately study day 9. Two consecutive negative stool cultures for *Shigella* are required before discharge (can be collected on the same study day). If the participant has not completed antibiotics, then the remaining doses of antibiotic will be given to the participant for self-administration. Vital signs will be collected.

4. Early Discharge

Early discharge is permitted in cases where early antibiotic treatment has been initiated. The participant needs 2 consecutive stool cultures negative for *Shigella* and to have taken two doses of antibiotic with resolved or resolving clinical symptoms before discharge. Remaining doses of antibiotic will be given to the participant for self-administration. Participants discharged before study day 8 will return on day 8 and provide the requisite samples (stool, blood) as delineated in the Schedule of Procedures.

5. Monitoring During Outpatient Phase

Participants will return to clinic 15, 29, 57, and 90 days after each challenge. They will also have a 6-month follow-up after the final challenge. Clinic visits during follow-up will include vital signs assessment, clinical checks, including concomitant medications and AEs, and sample collection for immunogenicity and exploratory outcome evaluations as per the schedule of events.

6. Concomitant Medications/Treatments

Only concomitant medications approved by the study physician will be used during the study. Participants needing to take unapproved or excluded medications will not be eligible for enrollment in this study. Any medication given during the trial (e.g., acetaminophen or antibiotics) will be documented in the participant's study chart and on the appropriate page of the electronic Case Report Forms (eCRFs). Approved medications that were being taken prior to, as well as during, the course of the trial will also be documented in this manner.

XI. STUDY SCHEDULE

1. Recruitment, Screening, and Enrollment of Study Volunteers:

Healthy participants will be recruited from the Baltimore, MD-Washington, DC region and surrounding areas (including other states) via IRB-approved advertisements, word of mouth, and contacting previous participants who have expressed interest in studies. Contact methods for this protocol include phone, electronic methods (including text and email), and mail. Study staff will respond to inquiries by phone, text, or email. Study staff will provide a brief, scripted synopsis of the research study that includes basic inclusion and exclusion criteria in compliance with the IRB-approved screening protocol. Participants who express interest in participating in the study will be asked to complete a telephone pre-screen to assess general health status and basic eligibility. Potential volunteers determined to be generally healthy and meeting basic eligibility requirements will be scheduled for an in-person screening. Participants will provide written informed consent prior to any study procedures.

2. JH200 Screening:

The CIR will use a screening protocol approved by the Johns Hopkins School of Public Health (JHSPH) IRB in recruiting participants for this study. The screening protocol is entitled "Screening of adult volunteers for eligibility to participate in clinical studies evaluating investigational vaccines, antimicrobial agents, or disease prevention measures or the pathogenesis of infectious agents," CIR 200, JHSPH IRB 00010083. Participants will be made aware that the screening process may take several visits to complete.

The following procedures will occur:

a) Discuss the study screening process and obtain Screening Informed Consent from the participant.
b) Ensure that the participant has a clear understanding of the nature of the screening study by independently passing a brief comprehension examination. Then ensure the participant signed and received a copy of the ICF.

c) Elicit a complete medical history, including menstrual and contraceptive history and/or history of surgical sterility for female participants.
d) Pregnancy prevention counseling for females focusing on the importance of preventing pregnancy during study participation, potential risks associated with pregnancy while taking investigational products, and effective contraceptive options.
e) Vital signs will be collected (heart rate, respiratory rate, blood pressure, and temperature).
f) Obtain blood for *Shigella* titers specific for *S. flexneri* 2a and *S. sonnei.*

Additionally, the following activities may occur or be deferred to a subsequent screening visit:

a) Administer a complete physical examination.
b) Provide HIV pre-test counseling, including that the testing is voluntary but necessary for study participation, information about HIV testing, transmission and prevention, explanation of test results, post-test counseling, and result reporting.
c) Obtain blood for a complete blood count (CBC) with differential, basic metabolic panel, ALT, AST, ABO blood typing, HLA-B27 antigen, IgA, Hepatitis B virus, Hepatitis C virus, and HIV testing. Obtain urine sample for toxicology testing, looking for the presence of amphetamines, barbiturates, opiates, phencyclidine, benzodiazepine, methadone, or cocaine metabolites. Screening CBC or chemistries may be repeated if there are potentially transient abnormalities or if there is concern for an error.
d) Females will have a serum sample taken for β-HCG testing.
e) Height and weight measurements.

3. Study-Specific Screening:

Approximately 110 healthy, potentially eligible and willing participants will be invited back to complete study-specific screening to allow for participants who do not continue to meet study eligibility criteria or wish to continue in the study. The following procedures will occur during this visit:

a) Participants will receive an overview of the study in an individual or group setting and an Informed Consent Form (ICF) to read on their own.
b) Participants will be encouraged to ask questions, and then independently complete a quiz to evaluate comprehension of study procedures, requirements, and risks. They must independently take and pass with ≥70% understanding. The participant will be able to retake the test once if they do not pass the first time. Study staff will meet individually with the participant to review the completed comprehension assessment. Incorrect answers will be reviewed with the participant.
c) Both the participant and staff member obtaining consent will sign, date, and time the ICF and a signed copy of the consent will be given to the participant.
d) Participants will read and agree to comply with the Inpatient Unit Guidelines, Alternate Agreement, and Handwashing Best Practice.
e) Volunteers will be asked to complete and sign a HIPAA medical record release form.
f) Female volunteers will receive pregnancy prevention counseling.
g) All participants will receive HIV pre-test counseling as described above, if not completed prior.
h) Inclusion and exclusion criteria will be assessed.
i) Demographics, medical history, including gender, date of birth, race, height, weight, and any allergies will be collected and/or reviewed.
j) Vital signs will be collected (heart rate, blood pressure, temperature).
k) Research samples will be collected as per schedule of events, if not already completed.
l) Complete physical exam will be done.
m) Functional bowel survey will be completed.

n) All volunteers will be tested for COVID-19 upon admission to the inpatient unit. Depending on the level of COVID illness in the community and current CDC and JHU guidelines, volunteers may be asked to obtain a COVID-19 test 1-5 days prior to admission. Any volunteer who tests positive for COVID-19 will not be eligible for admission.

o) As part of the pre-inpatient period, eligible subjects may be asked to perform a 5-minute baseline psychomotor vigilance test (PVT). Additionally, subjects will be provided a smartphone and actigraphy devices to be worn starting up to 21 days prior to challenge through the first post-discharge follow-up visit (study day 15 ± 2 days).

Attempts will be made to inform participants of their screening laboratory results either in person or over the telephone. Participants with clinically significant abnormalities (as determined by the PI or designee) may be asked to have additional blood drawn. If the result(s) is confirmed, participants may be referred to their primary care physician. A copy of the laboratory results may be provided to the participant at his/her request.

4. Enrollment

Participants will be considered enrolled in the study upon receipt of the Investigational Product.

5. Study Days

i. Day -2 or -1, Admission to the Inpatient Isolation Unit (Day 89 challenge 2)

Up to 34 volunteers who have provided informed consent and meet all eligibility criteria will be invited and 32 participants will be admitted to the inpatient unit (each admission). This is to allow for some dropout of the volunteers due to ineligibility or reversal of consent. Up to 30 participants each admission will be enrolled into the study and receive challenge as per Table 1. Volunteers who are admitted but not enrolled or do not receive challenge at that admission will be discharged and will not be followed.

On arrival to the unit, the following will be performed:

- Volunteer will be tested for COVID-19; volunteer must have a negative test on admission to be admitted to the inpatient unit.
- Volunteer will be admitted to the unit. Personal items will be reviewed. Unallowed items will be stored until discharge.
- Orientation to the unit.
- Vital signs (blood pressure, temperature, and heart rate) at least twice.
- Complete physical examination.
- Assessment for eligibility (inclusion and exclusion criteria).
- Concomitant medications will be reviewed.
- Serum pregnancy testing for females; if results are not available prior to challenge, urine pregnancy testing will be performed on Day 1.
- Blood will be collected for research, including immunological assays and serology.
- Blood will be collected for baseline assessments: CBC with diff, BMP, ALT and AST.
- Stool for qualitative and quantitative culture, and immunology (as per schedule of events and SSP). In the event a participant is unable to produce a stool on Day -2 or -1, at admission, stool may be collected any time on the day of admission or on Day 1, until 4 hours after challenge. Participant's inability to produce a stool on Day -2 or -1/Day 1 will not be exclusionary for challenge.
- Salivary IgA (as per schedule of events and SSP).
- Nasal swab for respiratory metagenomics (as per schedule of events and SSP).
- Pregnancy risk assessment and prevention counseling.

ii. Day 1, Challenge Day (Day 90 challenge 2)

- On the day of challenge, participants will receive a light breakfast and then initiate at least a 90-minute fast.
- Vital signs (BP, heart rate, and temperature) will be collected 4 times: prior to challenge, at least 30 minutes post challenge, and two additional times.
- A focused physical exam (symptom focused) and evaluation to assure that there are no changes from admission and ensure the volunteer remains eligible for challenge. Solicited and unsolicited AEs will be assessed.
- The alternates or a volunteer who does not continue to meet eligibility criteria will be discharged without challenge. Participants who never received a challenge dose will not be followed. Participants who previously received challenge will complete a Day 90 and Day 180 follow-up visit (as described in Section ix below).

iii. Challenge

- Approximately 1 minute prior to challenge (and within 2 minutes), participants will drink 120 mL of bicarbonate buffer (buffer formulation: 13.35 grams of sodium bicarbonate in 1000 mL of sterile water for irrigation).
- For challenge, participants will drink a solution of virulent *Shigella* bacteria (as per Table 1 and described in Section XII, 5), approximately 1500 cfu suspended in 30 mL of bicarbonate buffer (for 2457T strain) or saline (for 53G strain).
- Participants will be observed for at least 30 minutes for signs of adverse reactions, at which point their vital signs will be obtained and recorded 30-60 minutes after challenge.
- Participants will continue fasting for at least an additional 90 minutes post challenge.
- Following receipt of the *Shigella* challenge inoculum, all stools will be collected for weighing, grading and assessment for blood.
- If a participant develops grade 3 – 5 stools on Day 1, a sample of all grade 3 – 5 stools produced that day will be collected for possible culture. Up to 3 stools prior to the start of antibiotics should be sent for culture.

iv. Days 1 – 8, Planned Inpatient Days (Days 90-97 challenge 2)

- Vital signs (BP, heart rate, and temperature) at least 3 times a day; once in the morning, afternoon, and evening (approximately 6 hours apart).
- Participants will have a focused PE daily, with the assessment of any new or changes in solicited and unsolicited AEs.
- All medications will be provided by clinical staff and recorded on an inpatient medication administration form.
- All stools will be weighed, graded and assessed for blood.
- A minimum of one stool sample (no more than 3) will be collected daily for culture. If a volunteer is unable to provide a stool sample, rectal swabs will be obtained (as per SSP).
- Stool will be processed as per SSP daily while inpatient. (If the participant does not produce a stool or enough stool on any day to obtain all samples, this will not be considered a protocol deviation.)
- Blood and stool for immunological assessments will be collected as per the Schedule of Procedures.
- Saliva IgA collection (as per schedule of events and SSP).
- Nasal swab for respiratory metagenomics (as per schedule of events and SSP).
- Antibiotics will be started on Study Day 6 (5 days post challenge) unless early treatment criteria are met.
- Volunteers who have Grade 3 or greater adverse events may get safety labs (chemistries, CBC, liver functions tests) if a clinician feels that they are indicated.

- If subject develops low blood pressure despite IV hydration, the subject will be transferred to a higher level of care.

v. Day 9, Planned Discharge Day (Day 98 challenge 2)

Volunteers will be eligible for discharge when symptoms are resolved or are resolving, and they have had at least two consecutive stool cultures negative for the challenge strains (can be collected on the same study day) and have received at least 2 doses of antibiotics.

Volunteers will have:

- Focused PE prior to discharge
- Review of solicited and unsolicited AEs
- Receive morning dose of antibiotics (if course is not complete)
- Receive their additional doses of antibiotics to take after discharge
- At least one set of vital signs prior to discharge

Volunteers who do not meet criteria for discharge will remain on the unit and continue:

- Daily focused PE
- Vital signs three times a day
- Receive antibiotics (if not complete)
- Review of solicited and unsolicited AEs
- All stools will be weighed, graded and assessed for blood.
- Up to 3 stool samples and/or rectal swabs will be collected for culture

vi. Day 15 Outpatient Follow-Up Visit (+/- 2 Days) (Day 104 challenge 2)

- Interim medical history will be obtained
- AEs and SAEs will be recorded, including assessment of relatedness to challenge or antibiotics and severity grade
- Concomitant medications will be recorded
- Vital signs (BP, HR, and temperature)
- Focused PE if participant has any complaints/AEs
- Blood for safety (CBC w/differential and basic metabolic panel, ALT/AST)
- Blood and stool per Schedule of Procedures
- Saliva IgA collection
- Nasal swab for respiratory metagenomics (as per schedule of events and SSP)

vii. Day 29 Outpatient Follow-Up Visit (+/- 4 Days) (Day 118 challenge 2)

- Interim medical history will be obtained
- AEs and SAEs will be recorded, including assessment of relatedness to challenge or antibiotics and severity grade
- Concomitant medications will be recorded
- Vital signs (BP, HR, and temperature)
- Focused PE if participant has any complaints/AEs
- Pregnancy test (blood or urine; females only, to ensure that there was not an early pregnancy during the challenge period)
- Blood and stool per Schedule of Procedures
- Salivary IgA
- Nasal swab for respiratory metagenomics (as per schedule of events and SSP)

viii. Day 57 Outpatient Follow-Up Visit (+/- 4 Days) (Day 146 challenge 2)

- Participants will be questioned about their health status

- Adverse Events of Special Interest (AESIs) and SAEs will be assessed
- Vital signs (BP, HR, and temperature)
- Focused PE if participant has any AESIs and SAEs
- Blood and stool per Schedule of Procedures
- Salivary IgA
- Nasal swab for respiratory metagenomics (as per schedule of events and SSP)

ix. Day 90 (-7/+28 days) Outpatient Visit OR readmission to the inpatient unit for second challenge: (Day 180 challenge 2)

Participants who receive a second challenge will have their day 90 and day 180 after the second challenge. Unless the second challenge is >120 days after the first, then participants will have a day 90 outpatient visit and a day -2 or -1 visit inpatient.

Volunteers who receive the first challenge and are not selected for the second challenge will complete a **Day 90 outpatient visit**. This visit will include:

- Interim medical history
- AESIs and SAEs will be assessed
- Vital signs (BP, HR, and temperature)
- Focused PE if participant has any AESIs and SAEs
- Blood for assays as per Schedule of Procedures
- Nasal swab for respiratory metagenomics (as per schedule of events and SSP)

x. Re-Admission for Second Challenge

The volunteers in groups 1A or 2A will be assessed as to willingness and eligibility to participate in a second challenge (based on criteria Section IX, 4). Up to 21 volunteers who received the first challenge will be readmitted to the inpatient unit for second challenge, with the goal of challenging 20 (1 alternate will be admitted in case of loss of eligibility by a volunteer). If more than 21 volunteers from 1A or 2A are willing to be included in 1B or 2B 21 subjects will be randomly selected from the eligible subjects. Due to isolation unit constraints there need to be an even number of males and females to fill the 6 same-gender shared rooms. Additionally, up to 11 naïve volunteers will be admitted to the inpatient unit to receive challenge with the goal of challenging 10 participants.

- Participants will be questioned about their health status.
- AEs and concomitant medications will be assessed.
- Volunteers will be admitted to the unit. Items from home will be reviewed and items not allowed on the unit will be stored in the locked storage area for participant safety.
- Orientation to the unit.
- Vital signs (blood pressure, temperature, and heart rate) at least two times (at least 6 hours apart).
- Complete physical examination upon admission.
- Assessed for eligibility (inclusion and exclusion criteria).
- Serum pregnancy testing for females; if results are not available prior to challenge, urine pregnancy testing will be performed on Day 1.
- Blood will be collected for immunological assays and serology as per the Schedule of Procedures.
- Blood will be collected for baseline assessments: CBC with diff, BMP, ALT and AST.
- Stool for assays as per schedule of events and SSP. In the event a participant is unable to produce a stool on Day -2 or -1, at admission, stool may be collected at any time on the

day of admission or on Day 1, until 4 hours after challenge. Participants' inability to produce a stool on Day -2 or -1/Day 1 will not be exclusionary for challenge.
- Salivary IgA (as per schedule of events and SSP).
- Nasal swab for respiratory metagenomics (as per schedule of events and SSP).
- Pregnancy risk assessment and prevention counseling.

Prior to challenge, any alternate participants will be discharged from the unit prior to challenge. Those who had been previously challenged will receive follow-up as described below. The naïve alternates not previously challenged will not be followed-up after discharge. Participants who are readmitted and receive the second challenge will repeat the challenge schedule as described above in Section XI, 5.

xi. Day 180/270 Follow-Up Visit

All participants who receive at least one dose of challenge will complete a Day 180/270 follow-up visit. This visit will be approximately 180 days after their last challenge dose and will include:

- Functional bowel survey (e.g., Rome IV survey)
- Participants will be questioned about their health status
- AESIs and SAEs will be assessed
- Focused PE if participant has any AESIs and SAEs
- Blood for assays as per Schedule of Procedures
- Nasal swab for respiratory metagenomics (as per schedule of events and SSP)

6. Antibiotic Treatment

Routine antibiotic treatment will be given to all participants at about 5 days post-challenge. All participants will be treated with trimethoprim 160 mg / sulfamethoxazole 800 mg orally twice daily for five days, or ciprofloxacin (500 mg orally twice daily for three days), or, alternatively, ampicillin (500 mg) orally four times daily for three days.

Early antibiotic treatment after challenge **may** commence when any of the following criteria are identified **and** a study physician considers it to be warranted:
- When volunteer meets the primary endpoint (clinical definition of shigellosis)
- Confirmed oral temperature ≥ 39°C (after volunteer has not had anything to drink for about 20 minutes)
- Any other reason warranting early treatment in the physician's opinion

If, because of illness, a participant is unable to take oral antibiotics, intravenous antibiotics may be given at an appropriate dose based on weight and clinical status.

Once the volunteer has met the criteria for discharge, s/he will be released with the remaining antibiotic treatment to be taken at home. Volunteers taking any other non-antibiotic medication on a chronic basis may continue with that medication unless directed otherwise by the Investigator. These medications must be recorded on the appropriate eCRF.

7. Early Termination

Participants have the right to withdraw from the study at any time and for any reason withdrawal does not affect the responsibility of the investigator to treat the participant for study-related conditions. The investigator also has the right to withdraw the participants in the event of intercurrent illness, AEs, or for administrative/social reasons.

An excessive number of withdrawals can affect the scientific validity of the study; therefore, unnecessary withdrawal should be avoided. Should withdrawals occur, efforts will be made to ensure participant safety and continued monitoring as thoroughly as possible. In case of participant withdrawal, for whatever reason, every attempt to complete a final trial evaluation must be made stating the reasons. Withdrawals due to non-attendance must be followed-up by the investigator to the extent possible to obtain the reason for non-attendance.

Participants withdrawing from the study after receiving the *Shigella* challenge will receive antibiotics for outpatient treatment and will be educated on the importance of complying with treatment and good handwashing. Attempts will be made to follow the participant for clearance of the challenge organism through collection of stool samples and for safety through study day 29.

8. Laboratory procedures

Blood, stool, saliva and nasal swabs will be collected by trained personnel as per the Schedule of Procedures and as per SSPs. They will be labelled with the participant study numbers, processed and stored as per SSP on the inpatient unit or the clinic, and then packed and sent to either the CIR laboratory, WRAIR, Antigen Discovery Inc (ADi), IAVI or the University of Utah as per the SSPs and agreed upon shipment schedule. Research microbiology, including the preparation of the live inoculum and the culturing of stool specimens, will be carried out in the laboratory of the CIR in the JHSPH. Immunologic and other research assays (as per study schedule) will be carried out at the Bacterial Diseases Branch at WRAIR, the JHSPH CIR Enterics lab, Antigen Discovery, Inc (ADi) and the University of Utah.

Samples will be collected based on the Schedule of Procedures. Samples will be stored and distributed to various laboratories in compliance with DOT/IATA standards, guidance from the JHU Biosafety Review, and based on the SSP. Samples collected under this protocol will be used to conduct safety, microbiologic, and immunogenicity evaluations and other research labs (e.g., transcriptomics). Study-related samples will be collected and labeled according to the relevant SSP.

The following sections provide a listing of procedures and tests to be performed in this protocol at designated time points. The maximum volume of blood to be drawn will not exceed 550 mL over any 8-week period, which healthy adults should regenerate within this period and which should not compromise the health of trial participants.

Clinical Laboratory Tests:
Standard clinical laboratory tests to determine eligibility of participants and for safety monitoring will be carried out at Johns Hopkins Hospital, Johns Hopkins Bayview Medical Center, or Quest Diagnostics in Baltimore City, MD:

a) CBC with cell differential and platelets
b) Basic metabolic panel
c) Serum β-HCG
d) ALT/AST
e) HLA-B27 antigen testing
f) IgA testing
g) HIV assay (4rd generation)
h) HBsAg ELISA
i) HCV (3rd generation ELISA with immunoblot confirmation)
j) Urine for drug toxicology screening
k) ABO typing

As per the SSP the JHSPH research lab will perform the following test for screening:
S. flexneri 2a and *S. sonnei* 53G anti-LPS serum titer

Urine β-HCG and fecal occult blood tests will be performed by the CIR under a CLIA waiver.

9. Microbiology:

All microbiology of stool for *Shigella*, both quantitative and qualitative cultures, as well as possibly PCR, will be done at the JHSPH research lab.

10. Research Labs:

Blood, stool, saliva, and nasal swabs will be collected for research labs to be completed as per SSP at either the SPH research lab or at the Department of Diarrheal Research Laboratory at WRAIR, ADi, IAVI/Scripps and the University of Utah and may include, but is not limited to:

1. Blood for Anti-LPS serum IgG and IgA
2. Blood for serum IgG and IgA against *Shigella* antigens
3. Blood for total IgA and possibly IgG
4. Blood for alpha-4 beta-7 positive and negative cells and antibody from lymphocyte supernatant (ALS), memory B-cell assays
5. Stool for IgA against LPS and potentially Ipa proteins
6. Stool for inflammatory markers and transcriptomics/proteomics
7. Stool for microbiome
8. Stool for qPCR
9. Serology including bactericidal antibodies
10. Blood for transcriptomics and proteomics (systems biology)
11. Blood for antigen arrays (to be sent to ADi)
12. Blood for identification of potential broadly protective antibodies (IAVI/Scripps)
13. Saliva for salivary IgA against LPS and potentially Ipa proteins (to be processed in the laboratory of Chris Heaney, JHSPH)
14. Nasal swab for respiratory tract metagenomics (to be sent to the University of Utah)

11. Retention of Study Samples

Any unused blood, stool, saliva, or nasal swab specimens will be stored once the study is complete. Samples and data collected under this protocol may be used only for research purposes. No genetic testing will be performed on participant samples. These samples will not be sold or used to make commercial products.

Access to research samples will be limited using locked freezers. Samples and data will be stored using codes assigned by the investigators or their designee(s). Data will be kept on password-protected computers. Only investigators or their designee(s) will have access to the samples and data.

Any other research or experimental treatments will be conducted under other protocols for which separate IRB review and approval will be obtained.

During the study, samples may be sent to various research laboratories as per SSP. At the completion of the protocol (termination), samples that remain at JHSPH and data may be transferred to a repository (Johns Hopkins Bloomberg School of Public Health [JHSPH] IRB protocol R22.05.04.29.A2). Samples remaining at the Department of Defense will be maintained as per their SOP.

In the future, other investigators (both at the Johns Hopkins University (JHU), the DOD and outside) may wish to study these samples and/or data. In that case, IRB approval will be sought prior to any sample sharing. Similarly, IRB approval will be obtained prior to sharing any clinical information, with or without patient identifiers that may be linked to samples being shared. The research use of stored, unlinked, or unidentified samples (for example, as a standard for immunological analyses) may be exempt from the need for prospective IRB review and approval. Exemption requests will be submitted in writing to the JHU IRB office and to the PATH Research Determination Committee, as applicable, which are authorized to determine whether a research activity is exempt.

Any loss or unintentional destruction of samples, or data (for example, due to freezer malfunction, or missing case report forms [CRFs]) that compromises the scientific integrity of the study or poses a potential breach of confidentiality for the participant will be reported to the IRB.

Samples will be stored only with the participant's permission, which is a requirement for participation in the study. The participant may withdraw permission for future use of specimens at any time. If a participant withdraws his or her permission for future use of specimens, those specimens will be destroyed. In this case, the PI will ensure the destruction of all known remaining samples and will report this to both the participant and IRB. This decision may not affect the participant's participation in future protocols.

XII. STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

1. *Shigella* Challenge Inoculum/Product Characterization

S. flexneri 2a strain 2457T

The 2457T *S. flexneri* 2a strain is one of the strains that will be utilized in this study as a challenge. It is a well-characterized *Shigella* strain manufactured under current Good Manufacturing Practice conditions at the WRAIR Pilot BioProduction Facility (PBF) in Silver Spring, Maryland.

S. flexneri 2a strain 2457T was originally isolated from a patient with diarrhea in Tokyo in the fall of 1954 [17]. The organism was transferred to the WRAIR where it was used to produce a Production Cell Bank of strain 2457T under current Good Manufacturing Practices (cGMP) at WRAIR PBF. Validation and characterization of the 2457T MCB (lot 0589, BPR-321-00) is described in the BB-MF 3408 – Type II Master File – *Shigella* Species for Vaccine and Challenge (Live, Oral) (Serial No. 11 and Serial No. 14) held by the Office of the Surgeon General of the Army. The 2457T strain has since been used in several studies at the CIR [19], most recently in 2015 when 59 volunteers were challenged [26]. The *Shigella flexneri* 2a 2457T strain is susceptible to ciprofloxacin, trimethoprim-sulfamethoxazole and ampicillin.

Strain dose-finding studies have been completed for the 2457T *S. flexneri* 2a, and it will be given as approximately 1,500 cfu of 2457T *S. flexneri* 2a with a 90-minute fast in this study.

S. sonnei strain 53G

The 53G strain of *S. sonnei* was isolated in August 1954 in Tokyo, Japan, from a 5-yr-old male with diarrhea lasting for 2 days. The strain was described by Dupont et al in 1989 [21], confirming and establishing the low-dose virulence of 53G in humans. A master cell bank (MCB) (Lot 0593) was manufactured by the WRAIR PBF. The lyophilized 53G strain (Lot 1794) of *S. sonnei* was manufactured under current good manufacturing practices (cGMP) conditions at the WRAIR PBF.

Strain dose-finding studies have been completed for the 53G *S. sonnei* strain under BB IND 17015, held by PATH. The optimal dose and fasting times were determined from the outcome of a challenge development study conducted in Cincinnati by investigators at the Cincinnati Children's Medical Center. In that study, various doses of lyophilized bacteria were given to a total of 56 adult volunteers. A dose of approximately 1500 cfu resulted in 70% of the volunteers having diarrhea [32]. Based on those data, participants will be given approximately 1500 cfu of 53G *S. sonnei* with a 90-minute fast in this study. *S. sonnei* is sensitive to ciprofloxacin, trimethoprim-sulfamethoxazole, and ampicillin.

The disease manifestation of infection with orally administered wild-type *S. flexneri* 2a strain and *S. sonnei* 53G strain will be evaluated.

2. Packaging and Labeling

The challenge strains are stored as 1.0 ml aliquots (*S. flexneri* 2457T) with 1.8 x 10^8 cfu/mL or 2.0 mL aliquots of *S. sonnei* 53G with 2 x10^9 cfu/mL. The tubes are in cryostorage tubes held at -80°C +/- 10°C under controlled conditions at the WRAIR PBF. Labels of the strains are in Figures 2 and 3 below.

Figure 2: Production Cell Bank for 2457T *S. flexneri* 2a

Production Cell Bank for **2457T *S. flexneri* 2a** **BPR No.: BPR-322-01** **Lot No. : __1617_____** Contents: 1.0 ml Cautions: For Manufacturing Use Only; Viable organism Date of Mfg: 27 Jan 10 Manufactured By: WRAIR, Silver Spring, MD 20910

Figure 3: *Shigella sonnei* strain 53G

***Shigella sonnei* strain 53G** **BPR No.: BPR-1094-00** **Lot No. : ___1794_____** Contents: 2.0 ml Cautions: New Drug – Limited by Federal (or United States) law to investigational use Date of Mfg: 25 Feb 13 Manufactured By: WRAIR, Silver Spring, MD 20910

3. Product Acquisition, Storage and Transfer

S. flexneri 2a strain 2457T

The *Shigella flexneri* 2a 2457T challenge vials are stored at -80°C ± 10°C for short-term storage and in liquid nitrogen for long-term storage. The challenge strain will be transferred on dry ice from the WRAIR PBF to the CIR Enterics Research Laboratory at JHSPH, utilizing a temperature monitoring device, logged in and stored at -80°C ± 10°C in a locked and temperature-monitored freezer. Any use of these vials will be done under the supervision of the CIR Enterics Research Laboratory, JHSPH and tracked in an accountability log. Any vials remaining at the end of the study will be disposed of (via autoclaving) or returned to WRAIR for use in non-clinical research studies at the direction of study Sponsor and after all IP monitoring is completed.

Colony forming units (cfu) are not routinely monitored during frozen storage because the master cell bank is subcultured twice during inoculum preparation, and the total number of surviving organisms in the original frozen ampule is not critical. Nonetheless, strain stability is confirmed by subculture on tryptic soy broth (TSB)-containing Congo red dye since virulent *Shigella* bind this dye and grow as red colonies. In addition, the identity of isolated colonies used as a source of the inoculum for volunteers is confirmed as *S. flexneri* 2a by slide agglutination in type 2 antiserum.

S. sonnei strain 53G

The *S. sonnei* 53G vials are stored at -80°C (±10°C) freezer at the WRAIR PBF. Prior to administration, the requisite number of vials will be transferred to the research lab at the CIR Enterics Research Laboratory at JHSPH, logged in and stored at -80°C ± 10°C in a locked and temperature-monitored freezer. Any use of these vials will be done under the supervision of the CIR Enterics Research Laboratory, JHSPH and tracked in an accountability log. Any vials remaining at the end of the study will be disposed of (via autoclaving) or returned to WRAIR for use in non-clinical research studies at the direction of study Sponsor and after all IP monitoring is completed.

4. Product Preparation

The details of challenge strain preparation will be in the SSP. The challenge strains will be prepared by an experienced laboratory scientist under the supervision of an Investigational Drug Service Pharmacist.

S. flexneri 2a strain 2457T

For the 2457T *S. flexneri* 2a strain, fresh, plate grown organisms will be used for challenge inocula. Approximately 48 hours before challenge, a vial of the cGMP Master Cell Bank will be thawed. The GMP cell bank is plated onto 5 plates containing trypticase soy agar (TSA), to which Congo red (CR) dye (0.01%) has been added. The plates are streaked for isolation to yield well-separated colonies and then incubated at 37°C ± 1° C for 22 ± 2 hours. After incubation, six small, smooth, CR-positive colonies are tested for agglutination with commercial *S. flexneri* Type 2 antiserum (Denka Seiken Ltd., Japan). If these colonies give strong and rapid agglutination reactions, 10 well-isolated CR-positive colonies are picked and used to prepare a bacterial suspension in 3 mL of phosphate buffered saline (PBS). This suspension is used to inoculate multiple TSA plates to yield confluent growth (~6 plates for 30 volunteers, plus 2-3 extra plates as back-up in case of individual plate contamination). After growth for 22 hours at 37°C ± 1° C, the TSA plates are visually screened for purity, growth is re-checked for agglutination in antiserum and cells are harvested in sterile PBS (pH 7.4). For this step, 10 mL of PBS is added to each TSA plate and a sterile cotton swab is used to gently loosen the bacterial growth from the surface of the TSA plate. The bacterial suspension from each plate is collected with a sterile pipet, pooled and diluted in PBS to the target dose ($1.5x10^3$ CFU/ml) based on target OD_{600} values. The diluted preparation is administered to challenge study volunteers within 4 hours of preparation.

S. sonnei strain 53G

The day of challenge, vials will be removed from the freezer and placed on ice to thaw for 30 minutes. Then 2 mL of cold (2-8 ˚C) sterile water for injection (SWI) are added to each vial. To ensure complete rehydration of the challenge strain along with homogeneous mixing of the vial's contents, the vials remain on ice for another 15 minutes with intermittent swirling of the suspension. Vials will then be combined and diluted in cold, 0.9% USP sterile saline to arrive at the desired concentration. The diluted challenge material is kept on ice until administration. During reconstitution, an aliquot is removed for testing by colony count to be able to document the actual dose administered to the participants. The CFU total in the challenge inoculum that are

Form I is calculated according to study specific procedures and the reconstitution time is documented.

5. Product Administration

Participants will be challenged with approximately $1.5x10^3$ cfu of *S. flexneri* 2a strain 2457T or $1.5x10^3$ cfu of *S. sonnei* strain 53G.

Participants will fast for 90 minutes prior to ingesting the challenge inoculum. A sodium bicarbonate (USP-grade) solution of 2 g/150 ml water will be prepared. Each participant will drink 120 ml of this buffer one minute prior to ingesting the challenge inoculum, to neutralize gastric acidity. The bicarbonate buffer is prepared from USP-grade sodium bicarbonate by dissolving 13.35 grams of sodium bicarbonate in 1000 mL of sterile water for irrigation. Within 2 minutes, participants will drink the challenge inoculum (1.5×10^3 cfu) dissolved in either the remaining 30 mL of buffer (for the *S. flexneri* 2457T strain) or saline (for the *S. sonnei* 53G strain).

Participants will fast for an additional 90 minutes before resuming a normal eating pattern. Participants will be monitored for at least 30 minutes post challenge for any signs or symptoms of a reaction, and for any AEs including nausea and vomiting. If a participant vomits or does not ingest the whole dose, this will be documented; however, the participant will **not** be rechallenged or replaced. After at least 30 minutes, participants will have vital signs taken and will be free to move about the unit.

6. Accountability Procedures for the Investigational Products

The investigator must ensure that the investigational product is stored as specified in the protocol and in a secured area, with access limited to authorized study personnel. The investigator must maintain accurate records of the receipt of all investigational product, including date received, amount received and disposition. A record will be maintained that includes the dispensation date, amount of investigational product dispensed, initials and identification number.

Any use of the challenge vials will be done under the supervision and direct oversight of the Research Pharmacy and JHU Laboratory. The laboratory staff or Research Pharmacist will maintain a strain accountability log which tracks the status of all vials received. Any vials remaining at the end of the study will be returned to WRAIR or destroyed by autoclaving at the direction of study Sponsor and after all IP monitoring is completed. If destroyed, the investigator, in accordance with Sponsor's specifications, will document the destruction of any investigational product.

XIII. ASSESSMENT OF SAFETY

Safety monitoring will be conducted throughout the study; therefore, safety concerns will be identified by continuous review of the data by the PI, clinic staff, clinical monitor, research monitor, and the Sponsor.

Study Safety Management: The research monitor and PI will review any safety concern. A data safety monitoring board (DSMB) is not required for this study.

Research Monitor: A qualified physician with previous experience in vaccine biology and infectious diseases will be appointed as Research Monitor. Contact information, references and responsibilities of the Research Monitor will be included in the Safety Management Plan (SMP). Briefly, the Research Monitor will review any SAEs that occurred during the trial and any additional safety data

if necessary. In addition, the Research Monitor will provide guidance to the Principal Investigator on questions of eligibility and any question of participant safety.

The research monitor, in accordance with JHSPH guidelines, will have the following responsibilities:

- Evaluate ongoing safety data and make recommendations in order to ensure participants' safety as required
- Be available for consultation by the clinical investigative team through the period of the clinical study in which there is an interaction with human participants
- Be available to review all SAEs and other unanticipated problems involving risk to participants
- Provide clinical advice, in accordance with the study protocol, on the clinical management of participants. This advice may include, but is not limited to:
    - Decisions on participants who have "borderline" eligibility for enrollment
    - Confirmation and discussion of treatment decisions for difficult clinical situations

Protocol Safety Review Team (PSRT): The Protocol Safety Review Team (PSRT) will consist of the PATH Medical Officer, the PI and the Research Monitor and will be responsible for conducting periodic reviews of accumulating safety data and of trial conduct throughout the trial. Details on the responsibilities and processes of the PSRT, including periodicity of meetings and content of reports, will be provided in the Safety Management Plan. In addition to safety review, the PSRT may elect to discuss trial conduct issues that impact study integrity and/or participant safety. These may include, but are not limited to, data quality, critical monitoring findings, and issues with research specimens.

All clinical decisions must be documented, including date, time, and signature. All decisions must be communicated to the study PI and other study investigators, which must be stored with participant source documents.

All safety reports (i.e., SAEs, deviations, unanticipated problems involving risk and participant deaths) will be submitted to the JHSPH IRB and NMRC IRB per reporting requirements of each regulatory body.

1. Safety Monitoring-Vital Signs

Vital signs (temperature, blood pressure, and heart rate) will be obtained throughout the inpatient period and at study visits after discharge as per the study schedule (see Table 2 for applicable AE coding).

Table 2: Reference Ranges and Adverse Event Coding for Vital Signs Parameters

Vital Signs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Heart rate				
Tachycardia	101–115	116-130	>130	ER visit or hospitalization for arrhythmia
Bradycardia	50-54[a]	45–49	<45	ER visit or hospitalization for arrhythmia
Fever (°C) (°F)	38.0–38.4 100.4–101.1	38.5–38.9 101.2–102.0	>39.0 >102.0	Life threatening hyperthermia
Blood Pressure				
Hypertension (systolic, mm Hg)	141–150	151 - 155	>155	ER visit/hospitalization for malignant hypertension
Hypertension (diastolic, mm Hg)	91–95	96 – 100	>100	ER visit/hospitalization for malignant hypertension

Vital Signs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hypotension (systolic, mm Hg)[b]	85–89	80 – 84	<80	ER visit/hospitalization for hypotensive shock

[a]Grade 1 bradycardia will not be considered an abnormality for this study unless judged to be clinically significant by the PI or the PI in consultation with the Research Monitor and Sponsor.
[b]If a participant has a baseline systolic BP in the 90's then a decrease in BP < 10 without associated clinical symptoms will not be considered an abnormality for this study unless judged to be clinically significant by the PI.
Based on the FDA Guidance for Industry: Toxicity grading scale for healthy adult and adolescent volunteers enrolled in Preventative Vaccine Clinical Trials https://www.fda.gov/regulatory-information/search-fda-guidance-documents/toxicity-grading-scale-healthy-adult-and-adolescent-volunteers-enrolled-preventive-vaccine-clinical

2. Physical Examination

A complete physical exam will be conducted during the screening visit and on Day -2 or -1 as part of the screening process. PE to include HEENT (head, ears, eyes, nose, throat), skin, respiratory, cardiovascular, abdomen/GI, neurological, and musculoskeletal. Focused physical exams (symptom focused), will be conducted prior to receipt of any challenge product, daily during participants' inpatient stay, and as needed during outpatient visits with specific attention to the identification of AEs, AESIs, and SAEs.

3. Assessment of Stools

Participants will be asked to collect all stools passed on the unit and bring them to the lab for assessment and processing. Stools will be weighed and graded based on the following scale [3]:

- Grade 1, firm formed;
- Grade 2, soft formed;
- Grade 3, viscous opaque liquid or semiliquid which assumed the shape of the container;
- Grade 4, watery opaque liquid;
- Grade 5, clear watery or mucoid liquid.

Stools defined as grade 3, 4, or 5 are considered to be loose.
Stools will be observed for presence of gross blood; if a stool appears grossly bloody, it will be tested for blood using a hemoccult card. Stool that is not grossly bloody will not be tested for occult blood.

An episode of diarrhea will be considered complete after 24 hours without a loose stool.

During the outpatient period, loose stools will only be graded and assessed by study team based on the subject's description. No stools will be weighed during the outpatient period.

4. Laboratory Assessments

Venous blood samples will be collected for chemistry, hematology, and immunological parameters during the screening phase of this study, to provide a baseline sample, and after challenge as per the Schedule of Procedures. Hematology and chemistry analyses will be performed by a commercial laboratory. Additional specimens may be collected to confirm and evaluate any abnormal values and may be obtained as part of the clinical care of an individual participant. Targeted urine drug screenings are planned for this study at screening and urine or serological screening (including for surreptitious use of antibiotics) may be performed later at the discretion of the study clinician.

The clinical toxicity grading scale that will be used as a guideline is based on the FDA Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Participants enrolled in Preventive Vaccine Clinical Trials and the DAIDS Table for Grading the Severity of Adult and

Pediatric Adverse Events. Final grading determination will be made by the PI and according to Table 3 and Table 4.

In the event of a clinically significant abnormal laboratory value, the test will be repeated and followed up, if clinically relevant. Slightly abnormal laboratory values that remain consistent from the time of screening throughout the study will not be recorded as AEs.

Table 3: Reference Ranges and Adverse Event Coding for Clinical Hematology Parameters

Test	Normal	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (g/dL) (for screening purposes only)	M: LLN = 11.0 F: LLN = 10.5				
Hemoglobin, low		M: 10.0 to 10.9 F:9.5 to 10.4	M: 9.0 to <10.0 F: 8.5 to <9.5	M: 7.0 to <9.0 F:6.5 to <8.5	M: <7.0 F: <6.5
Eosinophils (cells/mm^3)	15-500	551-1,500	1,501-5,000	> 5,000	Hypereosinophilic
Leukocytes (white blood cells) (cells/mm^3)	2,500 to 10,800				
Leukopenia		2,000 to 2,499	1,500 to 1,999	1,000 to 1,499	<1,000
Leukocytosis		10,801-15,000	15,001-20,000	20,001-25,000	> 25,000
Lymphocytes, low (cells/mm^3)	>650	600 to <650	500 to <600	350 to <500	<350
Neutrophils, low (cells/mm^3)	>1,000	800 to <1,000	600to 799	400to 599	<400
Platelets decreased (cells/mm^3)	≥125,000	100,000 to 124,999	50,000 to <100,000	25,000 to <50,000	<25,000

Table 4: Reference Ranges and Adverse Event Coding for Blood Chemistry Parameters

Test	Normal	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
BUN (elevation)	7-25	26-28	29-31	> 31	Requires dialysis
Creatinine (elevation)	M: 0.7-1.4 F: 0.5-1.1	1.1 to 1.3 x ULN	> 1.3 to 1.8 x ULN OR Increase of > 0.3 mg/dL above baseline	> 1.8 to < 3.5 x ULN OR Increase of 1.5 to < 2.0 x above baseline	≥ 3.5 x ULN OR Increase of ≥ 2.0 x above baseline
Glucose, Random (mg/dL)	65 to115				
Hypoglycemia		55 to 64	40 to <55	30 to <40	< 30
Hyperglycemia		116 to 160	>160 to 250	>250 to 500	> 500
Potassium (mEq/L; *mmol/L*)	3.4 to 5.6				
Hypokalemia		3.0 to < 3.4	2.5 to <3.0	2.0 to <2.5	< 2.0
Hyperkalemia		>5.6 to <6.0	6.0 to <6.5	6.5 to <7.0	≥ 7.0
SGOT/AST (elevation)	M: 10 to 40 F: 10 to 35	1.25 to <2.5 x ULN	2.5 to <5.0 x ULN	5.0 to <10.0 x ULN	≥ 10.0 x ULN
SGPT/ALT (elevation)	M:9 to 46 F: 6 to 29	1.25 to <2.5 x ULN	2.5 to <5.0 x ULN	5.0 to <10.0 x ULN	≥ 10.0 x ULN

Sodium (mEq/L; *mmol/L*)	136 to145				
Hyponatremia		130 to <135	125 to <130	121 to <125	≤ 120
Hypernatremia		146 to <150	150 to <154	154 to <160	≥ 160

5. IND Safety Reporting - Definitions

The following terms, as defined by 21 CFR 312.32, apply to IND safety reporting.

Adverse Event

An adverse event is any untoward, undesired, or unplanned event in the form of signs, symptoms, disease, or laboratory or psychological/physiologic observations occurring in a subject enrolled in the clinical trial. This includes an exacerbation or worsening of pre-existing conditions or events, intercurrent illnesses, injuries, or vaccine or drug interaction, or worsening of abnormal clinical laboratory values. Anticipated day-to-day fluctuations of pre-existing conditions that do not represent a clinically significant exacerbation need not be considered AEs. Discrete episodes or worsening of chronic conditions occurring during a study period will be reported as AEs to assess changes in frequency or severity. Stable, pre-existing conditions and/or elective procedures are not AEs (including any not recognized or not reported prior to study entry) and will be recorded in the Medical History.

AEs will be documented in terms of signs and symptoms observed by the investigator or designee or reported by the participants at each study encounter, with a medical diagnosis stated. AEs occurring after informed consent is obtained, but prior to IP receipt, will be documented in the Medical History form within the participant's eCRF.

Suspected Adverse Reaction

A "suspected" adverse reaction means any adverse event for which there is a reasonable possibility that the investigational product caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the investigational product and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by an investigational product.

Solicited and Anticipated Adverse Events (Reactogenicity Events)

A solicited AE is a predetermined event, which may reflect safety concerns related to the IP. This study includes challenge with live *Shigella* bacteria, therefore all the symptoms of *Shigella* illness are expected. The most common effects of *Shigella* infection are listed below. These potential *Shigella*-associated AEs will be solicited daily during the challenge phase:

1. Abdominal cramps*
2. Abdominal pain*
3. Anorexia (poor appetite)
4. Arthralgias*
5. Bloating
6. Chills
7. Constipation
8. Excessive flatulence
9. Generalized myalgia*
10. Headache

11. Lightheadedness
12. Malaise*
13. Nausea*
14. Tenesmus
15. Urgency

*Constitutional and enteric symptoms that are in the definition of shigellosis

The following anticipated AEs will be documented via clinical assessments during the inpatient challenge phase:

1. Diarrhea of any severity (via stool logs)
2. Dysentery
3. Hypovolemia
4. Fever (oral temperature $\geq$ 100.4° F / 38.0°C)
5. Vomiting

Adverse Events of Special Interest

An AE of special interest is an AE of scientific and medical concern specific to the Sponsor's product or program, for which ongoing monitoring and rapid communication by the investigator to the Sponsor can be appropriate. Such an event might warrant further investigation in order to characterize and understand it.

AEs that are considered AESI are the following:

- Development of a new chronic illness
- Development of Reactive Arthritis
- Development of Irritable Bowel Syndrome or other continuing gastrointestinal condition

Serious Adverse Event

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

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

Although not considered SAEs, cancers will be reported in the same way as SAEs.

Pertinent definitions include:

- Life threatening - An AE is life threatening if the participant was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.
- Disabling/incapacitating - An AE is incapacitating or disabling if it results in a substantial disruption of the participants' ability to carry out normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as headache, nausea, vomiting, diarrhea injection site reactions and minor accidental trauma (e.g., sprained ankle).

- Hospitalization: In general, hospitalization signifies that the participant has spent significant time (usually involving at least an overnight stay) at the hospital or emergency ward for treatment that would not have been appropriate in the physician's office or outpatient setting. Hospitalization for either elective surgery related to a pre-existing condition which did not increase in severity or frequency following initiation of the study or for routine clinical procedures (including hospitalization for "social" reasons) that are not the result of an adverse event need not be considered as AEs and are therefore not SAEs.
- Routine Clinical Procedure: A procedure which takes place during the study and does not interfere with the test article administration or any of the ongoing protocol specific procedures.

Note: If anything untoward is reported during an elective procedure, that occurrence must be reported as an adverse event, either `serious' or non-serious according to the usual criteria. When in doubt as to whether 'hospitalization' occurred or was necessary, the AE will be considered serious. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or participant 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.

6. Relationship to Investigational Product (Assessment of Causality)

The investigator or designee must assign a relationship of each AE to the receipt of the IP. The investigator or designee will use clinical judgment in conjunction with the assessment of a plausible biologic mechanism, a temporal relationship between the onset of the event in relation to receipt of the IP, and identification of possible alternate etiologies including underlying disease, concurrent illness or concomitant medications. Every effort will be made to explain AEs and assess causal relationships, if any, to administration of the challenge, or antibiotic treatment.

AEs occurring after receipt of the challenge (Day 1) will be assessed as to their relationship with the *Shigella* challenge strain or the antibiotic, if applicable. The degree of certainty with which an AE can be attributed to these products (or alternative causes, e.g., natural history of the underlying diseases, concomitant therapy, etc.) will be determined by how well the event can be understood in terms of one or more of the following:

- Reaction of similar nature having previously been observed with *Shigella* challenge strains or antibiotic administration
- Published literature accounts supporting causality
- Temporal relationship with administration

The following guidelines should be used by investigators to assess the relationship of an AE to study product administration. Only a physician or nurse practitioner can make this determination. The investigator will assess causality of all AEs. Non-serious and serious adverse events will be evaluated as two distinct types of events given their different medical nature. If an event meets the criteria to be determined 'serious' it will be examined by the investigator to the extent possible to determine ALL contributing factors applicable to the event.

Other possible contributors include:

- Underlying disease
- Other medication

- Protocol-required procedure
- Other cause (specify)

Causality of all AEs will be assessed by the investigator using the following criteria:
In the investigator's opinion, the likelihood of the relationship of the AE to challenge administration is to be recorded as follows:

- Related: There is a reasonable causal relationship between the challenge strain administered and the AE.
- Not Related: There is no reasonable causal relationship between the challenge strain administered and the AE.

All AEs will be recorded on the appropriate AE form of the participant's eCRF and recorded irrespective of severity or whether or not they are considered related to the challenge inoculum. AEs will be tabulated separately for pre-and post-challenge data.

7. Recording Adverse Events – Method/Timing

AEs occurring from the time of consenting through 28 days after administration of Investigational Product will be recorded on the appropriate eCRF (AEs reported prior to challenge will be reported in the medical history eCRF). All AEs observed by the investigator or one of the clinical staff or reported by participants spontaneously or in response to a direct question will be evaluated by a study investigator or designee and recorded in the participant eCRF. The nature of each event, date of onset, outcome, severity and causality will be established. Details of any symptomatic/corrective treatment will be recorded on the source documents and entered in the appropriate page of the eCRF. AEs will be assessed as to their relationship with the challenge strain, the antibiotic (if treatment has started) or the protocol.

During the inpatient phase, both solicited and unsolicited adverse events will be assessed, whereas at outpatient visits only unsolicited AEs will be assessed. AEs already documented in the eCRF, i.e., at a previous assessment, and designated as 'ongoing' will be reviewed at subsequent follow-up assessments. If resolved, documentation in the eCRF will be completed. Changes in AE severity for solicited events during the inpatient period will be documented as a new event. Unsolicited AEs during the inpatient period, and any reported AEs with onset during the outpatient period, will be recorded as single events with the highest severity for the duration of the event entered in the eCRF.

Challenge-specific solicited AEs will be assessed during the inpatient phase of the study. Adverse events which occur after informed consent is obtained, but prior to challenge, will not be documented as AEs but as medical history, irrespective of severity. All AEs will be assessed through 28 days after each challenge, documented in the source records, and recorded on the eCRFs using accepted medical terms and/or the diagnoses that accurately characterize the event. AESIs and SAEs will be assessed through study completion (approximately 180 days from last challenge). Solicited AEs (listed above) will be recorded as individual events. Unsolicited AEs may be recorded as a diagnosis. When a diagnosis is known, the AE term recorded on the eCRF will be the diagnosis rather than a constellation of symptoms. The investigator will assess all AEs for causality, severity, and outcome. When an event has not resolved by the prescribed reporting period, it will be left open/without an end date on the AE eCRF and will be updated with end date or ongoing at the final visit.

8. Duration of Follow-Up of Participants after Adverse Events

Any SAE deemed related to IP that is ongoing at the time of last study visit will continue to be followed until it is resolved, assessed to be resolved with sequelae, or assessed to be

stable/chronic. SAEs deemed not related to IP that are unresolved at the time of the last study will be classified as ongoing. Investigators are required to follow related SAEs to resolution, even if this extends beyond the prescribed reporting period. Resolution is the return to baseline status or stabilization of the condition with the probability that it will become chronic. The SAE outcomes will be reported to the Sponsor.

Investigators are not obligated to actively seek SAEs in former participants; however, if a SAE considered to be related to the IP is brought to the attention of the investigator *at any time* until closure of the study, the event will be reported.

Investigators should follow-up adverse events at least until the final study visit. This may include repeat safety laboratory analysis. Outcome should be assessed as:

- Resolved
- Resolved with sequelae
- Severity change (the highest severity in a day will be recorded in the source documents; for solicited events during the inpatient period, if the severity on day 1 is moderate, then mild and then moderate, it will be entered as moderate for day 1 only, then if on day 2 the ongoing event is mild, the moderate AE will stop and the AE will be reentered as mild; unsolicited events that occur during both the inpatient and outpatient periods will be entered as single events to document the entire duration in the eCRFs, with the highest severity recorded for the event duration)
- Ongoing
- Died
- Lost to follow-up

9. Safety Assessments

All AEs will be assessed for severity by the investigator/designee. Inherent in this assessment is the medical and clinical consideration of all information surrounding the event including any medical intervention required. Each event will be assigned one of the following categories: mild, moderate, severe, or life-threatening. The criteria below may be used for any symptom not included in the grading scale. Any grade 4 (life-threatening) AE must be reported as an SAE.

For solicited AEs during the inpatient period, the eCRF will document daily changes for ongoing events. For any solicited AEs with onset during the outpatient period, the eCRF will reflect the highest severity for the entire duration of the event. Severity changes will be documented as described above.

Mild	Grade 1	Does not interfere with routine activities; minimal level of discomfort
Moderate	Grade 2	Interferes with routine activities; moderate level of discomfort
Severe	Grade 3	Unable to perform routine activities; significant level of discomfort
Potentially life-threatening	Grade 4	Hospitalization or ER visit for potentially life-threatening event

As defined by the International Conference on Harmonisation (ICH) guideline for Good Clinical Practice (GCP), the term “severe” is often used to describe intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself however, may be of relatively minor medical significance (such as severe headache). This is not the same as “serious”, which is based on participant/event outcome or action criteria usually associated with

events that pose a threat to a participant's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

During the challenge phase of the study, *Shigella* disease-specific adverse events will be graded in accordance with the table below.

Table 5: Challenge Inpatient Phase *Shigella* Infection Solicited Adverse Event/Endpoint

Reactogenicity/Adverse Event	Severity	Parameter
Diarrhea based on highest output of loose/liquid stools in any 24-hour period. (A diarrhea episode ends when there is a 48 hour window with no grade 3-5 stools.)	1- Mild	≥ 2 loose / liquid stools weighing ≥ 200g within 48 hrs. or 1 stool weighing ≥300g, not meeting the definition for moderate or severe
	2- Moderate	4 to 5 loose/liquid stools within 24 hours or 401 - 800g of loose/liquid stool within 24 hours
	3- Severe^	≥6 loose/liquid stools within 24 hrs. or >800g of loose/liquid stool within 24 hours
	4	Potentially life threatening
Oral temperature* (t)	1- Mild	≥100.4ºF and ≤101.1ºF (38.0-38.4ºC)
	2- Moderate	≥101.2ºF and ≤102.0ºF (38.5-38.9ºC)
	3- Severe	>102.0ºF and ≤ 104 ºF (39.0 - 40ºC)
	4	> 104 ºF or 40ºC Life threatening hyperthermia
Vomiting	1- Mild	One episode within a 24-hour period
	2- Moderate	Two episodes within a 24-hour period
	3- Severe	Three or more episodes within a 24-hour period
	4	Potentially life threatening consequence of emesis
Other solicited and non-solicited adverse events	1- Mild	Discomfort noted, but no disruption of normal daily activities; slightly bothersome; relieved with or without symptomatic treatment.
	2- Moderate	Discomfort sufficient to reduce or affect normal daily activity to some degree; bothersome; interferes with activities, only partially relieved with symptomatic treatment.
	3- Severe	Discomfort sufficient to reduce or affect normal daily activity considerably; prevents regular activities; not relieved with symptomatic treatment.
	4	Potentially life threatening

*Oral temperature; no recent hot or cold beverages, eating or physical activity. If temperature is ≥ 100.4 °F every attempt should be made to repeat the temperature in about 20 minutes after the participant has rested with no eating or drinking. If the repeat temperature is WNL and the investigator feels the repeated temperature is a more accurate reflection of the participant's real temperature a fever will not be entered into the eCRF. ^A subset of more severe diarrhea is ≥10 loose (grade 3-5) stools within 24h or ≥1000 gr loose (grade 3-5) stools within 24h.

Table 6: Classification of Intensity of Diarrhea during the Outpatient Phase

Diarrhea	**Mild (Grade 1)**	**Moderate (Grade 2)**	**Severe (Grade 3)**	**Potentially Life Threatening (Grade 4)**
Loose stools are stools to be determined to be grade 3 - 5 by study team	3 loose stools in 24 hours	4-5 loose stools in 24 hours	6 or more loose stools in 24 hours	Requires hospitalization

10. Reporting Adverse Events

The PI will report all AEs as per regulatory requirements.

11. Outcome Adjudication Committee

To obtain an unbiased determination of the efficacy outcomes, an independent outcome adjudication committee will blindly evaluate challenge outcome data after completion of all

inpatient phases of the study. To ensure a blinded review, new subject IDs that differ from those at assigned at enrollment will be utilized.

The committee will be comprised of at least 3 individuals, independent of the study Sponsor and investigative team, who are experts on diarrheal illness case identification and pathogen diagnosis. The committee will also include a statistician data analyst who will lead and coordinate the committee but will have a non-voting role in deliberations.

The committee voting members will review all potential efficacy-related cases and endpoint data. Among the committee's responsibilities, they will review and confirm all primary endpoint cases and 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 in the manual of procedures (MOP) prior to the start of the study.

12. Reporting Serious and Unexpected Adverse Events

SAE reports will be provided to the Sponsor, research monitor, and IRB(s). The investigator must report SAEs within one calendar day of becoming aware of the event by telephone, fax or e-mail (if appropriate) to the contacts in Table 7 below for reporting SAEs as described in the protocol. This initial notification will include minimal, but sufficient information to permit identification of the reporter, the participant, the test articles, SAE(s), and date of onset. The investigator will not wait for additional information to fully document the event before notifying. The first notification will be confirmed by an acknowledgement by email from the Sponsor, research monitor, and IRB. The report is then to be followed by submission of a completed SAE Report Form as soon as possible but not more than 3 calendar days past the initial report, detailing relevant aspects of the SAE in question. All actions, if any, taken by the investigator regarding the SAE and event outcomes must also be reported immediately. SAE Report Forms are to be used for documentation of these various aspects regarding the event. Hospital records and autopsy reports will be obtained if applicable.

The research monitor is required to review all unanticipated problems involving risk to participants or others, SAEs, and provide an unbiased written report of the event. At a minimum, the research monitor will 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 will indicate concurrence or nonconcurrence with the details of the report provided by the investigator. Reports for events determined by either the investigator or research monitor to be related to participation and reports of events resulting in death will be based on regulatory guidance.

Unanticipated problems involving risk to participants or others, SAEs related to participation in the study and all participant deaths will be reported by phone, by email, or by facsimile. A complete written report will follow the initial notification.

13. Reporting to the Sponsor

All SAEs must be reported promptly to the Sponsor as per 21 CFR 312.64, whether or not the event is considered related to study product. Further, the investigator should comply with relevant study site SOPs on reporting SAEs. The minimum information that the investigator will provide to the Sponsor is specified in Table 8.

Table 7: Study Contacts for Reporting Serious Adverse Events

Sponsor	PATH Patricia Njuguna, MD ACS Plaza, 4th floor Lenana Road P. O. Box 76634-00508 Nairobi, Kenya
Institutional Review Board	JHSPH IRB Office 615 N. Wolfe Street, Suite E1100 Baltimore, Maryland 21205 Phone: 410-955-3193 Toll-Free: 1-888-262-3242 Fax: 410-502-0584 Email: JHSPH.irboffice@jhu.edu
Research Monitor	Anna P. Durbin, MD Professor, International Health Johns Hopkins Bloomberg School of Public Health 624 N. Broadway, Room 251

Table 8: SAE Information to be Reported to the Sponsor

Notification Method	**Information to be Provided**
Email or Telephone (within 72 hours)	IND number, Sponsor study number, name of the IP, investigator name and contact number
	Participant identification number
	SAE, onset date, date of IP administration, severity, relationship, and participant's current status
AND	
Email or Fax	Cover sheet or letter
	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
NOTE: When submitting SAE reports via email, the subject line of each email notification will read as follows: **SAFETY REPORT – IND # _____, Study #_____, Participant# _____, Event term: _____**	

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

Investigators must follow all relevant regulatory requirements as well as specific policy at each institution regarding the timely reporting of SAEs to the local IRB and research monitor. Reporting to the Sponsor does not fulfill the investigator's duty to report all unanticipated problems involving risk to human participants or others to the IRB. The PI will notify the local IRB and the research monitor.

14. Reporting to the IRB

Unanticipated problems involving risk to participants or others, SAEs related to participation in the study and all participant deaths should be promptly reported by phone, email, or fax to the

local JHSPH IRB and protocol associated IRBs as needed. A written report will follow the initial notification.

Investigators are required to forward safety information provided by the Sponsor's representative to the IRB. All SAEs will be reported to the JHSPH IRB according to IRB guidelines.

JHSPH IRB contact: Phone 410-955-3193; Fax 410-502-0584; e-mail: jhsph.irboffice@jhu.edu. Investigators are required to promptly report adverse events that fit the following criteria using the Problem/Event Report Form:

Event (including adverse event reports, injuries, side effects, breaches of confidentiality, or other problems) that occurs any time during or after the research study, which in the opinion of the principal investigator:

1. Involved harm to one or more participants or others, or placed one or more participants or others at increased risk of harm;
2. Is unexpected (an event is "unexpected" when it is not described with specificity in the protocol and informed consent document; or if described with specificity, it occurs beyond the expected frequency and/or severity identified); and
3. Is related to the research procedures (an event is "related to the research procedures" if in the opinion of the principal investigator, it was more likely than not to be caused by the research procedures.)

15. Immediately Reportable Events - Pregnancy

A serum sample for pregnancy testing (female participants) will be collected at the screening visit and on Day -2 or -1. A urine pregnancy test will be collected (female participants) on Day 1 only if serum pregnancy test results are not available prior to challenge, and Day 29. Any participant with a positive pregnancy test prior to any challenge will not be enrolled in the study. Any participants who become pregnant during the study will be removed from the study and followed until the end of their pregnancy.

Each pregnancy must be reported **within 72 hours of identification** by email or fax to the Sponsor and the IRB. The investigator must report any pregnancy in study participants to the Research Monitor within 14 calendar days of learning of this occurrence.

Participants who become pregnant after Day 1 through 29 days after each challenge dose will be followed to term, and the following information will be gathered for outcome: date of delivery; health status of the mother and child, including the child's gender, height, and weight. Complications and/or abnormalities should be reported, including any premature terminations. A pregnancy is reported as an AE or SAE only when there is suspicion that the IP may have interfered with the effectiveness of contraception or there was a serious complication in the pregnancy, including a spontaneous abortion or an elective termination for medical rationale.

Pregnancies that occur after 29 days after each challenge will not be followed.

16. AE-Related Withdrawal of Consent

Any AE-related withdrawal of consent during the study must be reported *immediately* **(within 24 hours of identification)** by email or fax to the Sponsor and the IRB.

17. Pending Inspections/Issuance of Reports

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

The investigator will notify the Sponsor within 24 hours following contact by a regulatory agency. The investigator and study coordinator will be available to respond to reasonable requests and audit queries made by authorized representatives of regulatory agencies. The investigator will provide the Sponsor with copies of all correspondence that may affect the review of the current study or his/her qualification as an investigator in clinical studies conducted by the Sponsor. The Sponsor will provide any needed assistance in responding to regulatory audits or correspondence. The investigator will permit independent auditors (employees of the Sponsor or an external company designated by the Sponsor) to verify source data validation of the regularly monitored clinical trial. The auditors will compare the entries in the eCRFs with the source data and evaluate the study site for its adherence to the clinical study protocol and GCP guidelines and applicable regulatory requirements.

18. IND reporting

Annual

The lead investigator will be responsible for the preparation of a detailed annual synopsis of clinical activity, including adverse events, for submission to the Sponsor. Each annual report will summarize IND activity for 1 year beginning approximately 3 months before the IND FDA anniversary date.

Final

A final study report will be prepared in accordance with "Guidance for Industry: Submission of Abbreviated Reports and Synopses in Support of Marketing Applications" and ICH E3 Guideline "Structure and Content of Clinical Study Reports" and provided to the Sponsor for review and approval. The Sponsor representative will use this report to prepare the final clinical study report for submission to the FDA. The investigative team will report all AEs to the Sponsor and the local IRB in the appropriate safety, annual, and/or final reports.

19. Safety Criteria for Stopping Further Challenges

The PI, along with the research monitor, may determine if certain events warrant discontinuation of challenge for all participants in a cohort. If any of the following events occur, administration of the challenge will be discontinued for all participants in that cohort, and the PI and the research monitor will undertake a thorough review of the events:

- The occurrence of one or more serious adverse events (SAEs) determined to be related to the challenge.
- Systemic allergic reaction, including but not limited to generalized urticaria, generalized petechiae, or erythema multiforme, occurring in two or more participants in a group. Bronchospasm or anaphylaxis occurring in any participant.

Based on prior experience with *Shigella* challenge studies, it is expected that some participants will have severe AEs (such as severe diarrhea).

AEs which will prompt stopping the challenge for an individual participant include:

- The investigator deems that stopping test article administration is in the best interest of the participant.

Further challenge, in accordance with the protocol, may be resumed with the concurrence of the research monitor, Sponsor, and PI.

20. Study Termination

The PI, Research Monitor, IRB, Sponsor or FDA may stop or suspend the use of either or both challenges at any time. Suspension or termination of research must be reported to the PATH Office of Research Affairs (ORA) within 72 hours.

XIV. STUDY MONITORING

1. Monitoring

Monitoring shall be conducted by a designated contract research organization according to an approved monitoring plan that will be finalized prior to the start of the study. Local monitoring will commence at study initiation, during the study, and at closeout.

The study monitor shall be available for consultation with the investigator. The study monitor or other authorized representatives of the Sponsor may inspect all documents and records maintained by the investigator, including, but not limited to, medical records (office, clinic or hospital) and pharmacy records for the participant in this study. The clinical study site will permit access to such records. The investigator will obtain, as part of informed consent, permission for authorized representatives of the Sponsor, or regulatory authorities, to review, in confidence, any records identifying individuals in this clinical study.

2. Data Entry

Source documents will be maintained in real-time. Data from the source documents will be entered into the database as per the Data Management Plan. Data entry will commence when the last participant is discharged from the inpatient unit. Priority data points may be entered for all participants first before remaining data. Outpatient visits will be completed, and data will be entered into the database within about 3 working days.

3. Data Management/Collection/Source Documents

The investigator will maintain complete and accurate documentation of the study, including records of medical treatments external to the research received during the study, records detailing the progress of the study for participants, laboratory reports, source documents, signed informed consent forms, investigational product disposition records, correspondence with the IRB, the study monitor and the Sponsor, AE reports, and information regarding participant discontinuation and completion of the study. All required data will be clearly and accurately recorded by authorized study personnel in the eCRFs. Only designated study site personnel will record or change data in an eCRF. The investigator will be responsible for procuring data and for quality of data recorded in the eCRFs. Complete source documentation (the documents in which data collected for the clinical trial are first recorded) is kept for each participant in his/her individual study chart. All laboratory specimens, reports, study data collection, and administrative forms will be identified by coded number only to maintain participant confidentiality. eCRFs using coded identifiers will be used to record data for participants enrolled in the study. Forms, lists, logbooks, appointment books, and any other listings that link participant ID numbers to other identifying information will be stored in a separate, locked file in an area with limited access. All information regarding study participants is kept in password-protected computer files or in locked file cabinets that can be accessed only by authorized study personnel. Samples are identified by coded participant numbers only. Chart information and information from study records is not released without written permission of the volunteer.

The source documents will be retained at the site. All study-related documents will be kept in locked cabinets in locked rooms with limited access. Information in the electronic database is password-protected and access is available only to authorized research team members. Additionally, each authorized research team member is assigned a level of security clearance (also password-protected, with mandatory password changes) for the purpose of limiting access to certain areas or functions of the database. Any information printed from this database is stored in locked files until its use is complete and then shredded.

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

A detailed data management plan (DMP) will be developed by the designated contract research organization and approved by the study team, the PI, and the Sponsor. The DMP will explain how all AEs will be coded. Additionally, a data dictionary and metadata report will be compiled to explain the values that can be entered into the study database.

XV. STATISTICAL CONSIDERATIONS

1. Introduction

Safety, efficacy, and clinical outcome data will be entered into the eCRFs using standard software for data management. Data will be reviewed and errors corrected with standard strategies for range and consistency checks. AEs for all participants, regardless of the number of challenge doses they have received, will be included in the safety analysis. The null hypothesis for this study is that there is no protection provided by the initial challenge upon rechallenge. Immunology data will be imported after appropriate immunological testing has been completed.

2. Volunteer Selection

Volunteers will be recruited from the community as per Sections XI and XX. Volunteers will be recruited sequentially into each Cohort/Group. Cohort 1 is defined as the collection of individuals who are challenged with *S. sonnei* first followed by *S. flexneri* 2a challenge; Cohort 2 is the collection of individuals who are challenged with *S. flexneri* 2a first followed by *S. sonnei*. A group is the subset of the cohort that is admitted to the unit at any given time (4 groups, 1A, 1B, 2A, and 2B). If more than 21 volunteers from 1A or 2A are willing to be included in 1B or 2B, 21 subjects will be randomly selected from the eligible subjects. Due to isolation unit constraints, there needs to be an even number of males and females to fill the 6 same-gender shared rooms.

3. Definitions of populations to be analyzed

Safety population

All subjects who have been screened, consented, and proceeded to receive the challenge strain. This population will serve as the primary analysis population for the safety endpoints.

Per protocol population

All participants in the Safety population who have no major protocol violations that are determined to potentially interfere with the immunogenicity assessment. This population will serve as the primary analysis population for the immunogenicity endpoints. The population will be adapted by time point to include all eligible subjects' data up to the time of the disqualifying protocol deviation. Membership in this study population will be determined in a blinded fashion.

4. Analysis

A statistical analysis plan (SAP) for this study is in development and will be finalized prior to database lock. Safety and immunogenicity populations will both be comprised of subjects who have been screened, consented, and proceeded to receive the challenge strain. Missing data are expected to be minimal, no imputation will be performed for missing values and data will be assumed to be missing at random. SAS version 9.4 (Cary, NC) will be used to complete study analyses. Due to the descriptive nature of this study no adjustments for multiple testing will be performed.

Baseline Data

Baseline data such as demographics, medical history, as well as disposition of the volunteers will be summarized in tables or text in the final study report, as per the details in the SAP.

Rates of Shigellosis

Each day of the inpatient period, subjects will be monitored for loose stools (grade 3-5 based on assessment by the clinical team that do not meet the study's diarrhea definition), diarrhea, nausea, vomiting, abdominal pain or cramps, fever, headache, myalgia, arthralgia, and malaise along with the other solicited symptoms listed above.

Attack rates of shigellosis will be calculated for all study groups using the standard definition as follows: (# with endpoint / # receiving inoculum) x 100%. These attack rates will be estimated along with 95% confidence intervals (both asymptotic estimates and exact) for each study group. Attack rates between *Shigella* naïve participants and previously challenged participants will be compared using a Pearson's chi-squared or Fisher's exact test, as appropriate.

Safety Analyses

All AEs will be summarized and compared between groups. Safety data, including reactogenicity, AEs, stool information, specified vital signs, abnormal physical exam findings and laboratory tests will be listed by study subject. This information will be summarized in data tables as per the SAP. Additionally, tables will be prepared to list each commonly observed AE, the number of subjects who experienced an event at least once, and the rate of subjects with AEs. All AEs will be divided into defined severity grades (mild, moderate, severe, and life-threatening). There will be separate summaries of SAEs, as well as severe AEs, AEs of special interest, SUSARs, etc. should they occur.

Descriptive statistics (n, mean, standard deviation, median and ranges for continuous variables, percentages for categorical variables) will be compiled for each group.

Immunology Analyses

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 95% confidence intervals. Geometric mean titers will also be determined and presented with their 95%

confidence intervals. For each assay, the fold-increase will be considered a response, or the threshold for responders or non-responders will be pre-determined if possible. This will be described in greater detail in the SAP.

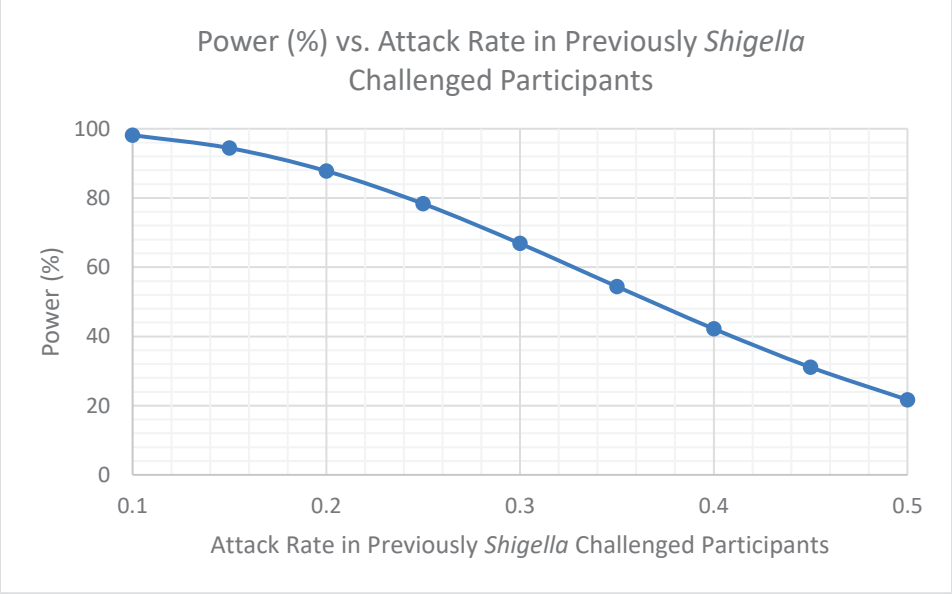
Comparisons across groups of single immune parameters reported as continuous variables (e.g., reciprocal log10 titers) will be made utilizing t-tests and one-way analysis of variance, Wilcoxon rank-sum, or Kruskal-Wallis H tests, as appropriate. Comparisons of responses within groups over time will be made utilizing appropriate paired analyses. Immune parameters that are nominal in nature (e.g., responder status) will be compared using chi-squared or exact tests, as appropriate. Additionally, comparisons of immune response profiles, assessing multiple immune parameters simultaneously, will be explored utilizing multivariable regression models, principal component analyses, and/or other multivariable methods, as appropriate.

All hypothesis testing will be conducted using a 2-sided *alpha*=0.05.

Further details of the statistical analysis plan for secondary and tertiary endpoints, if relevant, will be included in the SAP.

5. Sample Size Considerations

Presuming a shigellosis rate of 70% in naïve participants, a sample size of 16 participants will yield 95% confidence intervals (asymptotic estimates) of 48-92%, while a sample size of 8 participants will yield 95% confidence interval (asymptotic estimates) of 38-100%. Presuming an ability to pool naïve participants (if the cohorts have a comparable attack rate), a sample size of 19 participants in the previously *S. sonnei* 53G challenged arm and 16 participants in the *S. flexneri* 2457T *naïve arm* provides a >80% power to detect a shigellosis risk difference of 46% presuming a ≥70% attack rate in *Shigella* naïve participants, while a sample size of 11 participants in the previously *S. flexneri* 2457T challenged arm and 8 participants in the *S. sonnei* 53G naïve arm provides a >80% power to detect a shigellosis risk difference of 60% presuming a ≥70% attack rate in *Shigella* naïve participants (based on a Pearson's chi-squared test for proportional differences and a 2-sided *alpha*=0.05). A power curve, corresponding to a sample size of 19 participants in the previously *S. sonnei* 53G challenged arm and 16 participants in the *S. flexneri* 2457T naïve arm and an attack rate of 70% in *Shigella* naïve participants is provided below.



XVI. OBLIGATIONS AND ROLES OF THE SPONSOR, INVESTIGATOR, AND STUDY PERSONNEL

This study will be conducted using GCP and in accordance with all federal regulations regarding the protection of human participants in research including The Nuremberg Code, The Belmont Report, US 21 CFR Part 50 – Protection of Human Subjects, 32 CFR 219 (The Common Rule) and all regulations pertinent to the Department of Defense.

The investigators agree to conduct the research in strict accordance with this protocol, the ICH Guideline for GCP (CPMP/ICH/135/95), as well as in conformity with any federal, provincial or local regulations regarding the conduct of clinical studies. The Sponsor and investigator must comply with all applicable regulations. In addition, the investigator must follow local and institutional requirements including, but not limited to, IP, clinical research, informed consent and IRB regulations. The Sponsor will provide notification to the investigator of protocol and amendment approvals by regulatory authorities when applicable. Except where the investigator's signature is specifically required, it is understood that the term "investigator" as used in this protocol and on source documents refers to the investigator or appropriate study personnel that the investigator designates to perform a certain duty. The investigator is ultimately responsible for the conduct of all aspects of the study. Sub-investigators or other appropriate study personnel are eligible to sign for the investigator on designated source documents.

All amendments to the protocol, consent form and/or any IRB approved documents, including a change of PI, will be submitted to the Sponsor and IRB(s) for review and approval prior to implementation.

XVII. QUALITY CONTROL AND ASSURANCE

During the study, the investigator will maintain complete and accurate documentation of the study, including records detailing the progress of the study for each participant, laboratory reports, eCRFs, signed informed consent forms for each study participant, investigational product disposition records, correspondence with the IRB, the study monitor and the Sponsor, adverse event reports and information regarding participant discontinuation and completion of the study. All required study data will be clearly and accurately recorded by authorized study personnel in the eCRFs. Only designated study site personnel shall record or change data in an eCRF. During the study, the investigator will be responsible for the procurement of data and for quality of data recorded in the eCRFs. The study monitor will ensure accuracy of the eCRFs.

XVIII. PROTOCOL DEVIATIONS

Major protocol deviations are a subset of protocol deviations that may significantly impact the completeness, accuracy, and/or reliability of the study data or that may significantly affect a subject's rights, safety, or well-being. Non-emergent/minor deviations are routine departures that typically involve a participant’s failure to comply with the protocol. Examples: missing scheduled visits; failing to complete required questionnaire.

Other-than-minimal-risk changes and all unanticipated major problems involving human subjects or others will be reported promptly to the IRBs, and no such changes will be made to the research without IRB approval unless necessary to eliminate apparent immediate hazards to human subjects.

Minor minimal-risk deviations necessitated during the course of the trial will be made on site as needed and documented for subsequent review within a reasonable time period. Deviations from the protocol that potentially impact participant safety will be promptly reported to the Research Monitor, IRB, and PATH ORA. Other deviations will be reported at the time of continuing review.

XIX. PROTECTION OF HUMAN SUBJECTS

1. Insurance and Indemnity

There are limited funds available to the study for care of study-related injury. Although the investigator and Sponsor will make every effort to cover the costs of any study-related injury, full coverage cannot be guaranteed, and uncovered costs may fall on participants or their insurers. Therefore, participants will be informed that there are limited funds to cover study-related injury care costs and that there is the possibility that they may ultimately be responsible for at least some of the cost of care.

The Sponsor will obtain and maintain appropriate and commercially reasonable amounts of insurance, including commercial general liability insurance, and product and completed operations liability insurance, including coverage for the clinical trial.

2. Risks/Benefits

Risks Associated with *Shigella* Infection**:** Naturally-acquired illness caused by *S. sonnei* and *S. flexneri* 2a ranges from mild-to-severe watery diarrhea that may contain mucus or blood. Nausea, vomiting, abdominal cramping, headache, abdominal gurgling or gas, anorexia, fever, muscle and/or joint aches, and malaise may occur. For most adults, the illness is not life threatening but often leads to mild-to-moderate dehydration and significant inconvenience associated with loss of sleep and activity. Study facilities will have personnel and resources capable to manage diarrheal illness and potential complications.

Potential risks associated with *Shigella* infection/challenge are not limited to the acute effects of disease, but might extend to post-infectious conditions, like reactive arthritis (characterized by symptoms including inflamed joints and inflammation of the genital, urinary, or gastrointestinal systems) or irritable bowel syndrome (IBS) (23). In order to limit the risk of post-infection conditions, history of autoimmune disease or inflammatory arthritis and positive tests for HLA-B27 (a marker associated with reactive arthritis) are checked at screening as exclusion criteria. In addition, a functional bowel disorder survey will be conducted at screening and at Day 180/270. Participants with reported prior history of abnormal bowel patterns who might be at higher risk of this post-infectious sequelae will be excluded.

Risks Associated with Transmission of Challenge**:** The *Shigella* strains have the potential for risks to both the environment and to the research personnel; however, the risk to the environment in regard to potential transmission outside of the CIR facility is low. There is a minimal risk of acquiring *Shigella* infection associated with participant inoculum administration, patient care activities on the ward, or processing *Shigella*-infected stool. The risk to the environment will be reduced by ensuring that all human waste products from inpatients are disinfected with bleach prior to disposal, ensuring all participants comply with discharge criteria (two consecutive negative stool cultures for *Shigella*), emphasizing the importance of handwashing for participants and staff, ensuring proper disposal/cleaning of linen, and cohorting participants in the CIR while shedding *Shigella*. Participants will be instructed as to the importance of completing the 3- or 5-day course of antibiotics and this instruction will be documented.

Risks Associated with Antibiotics**:** Therapeutic antibiotics for use in this study are licensed, approved medications that have been used extensively, and shown to be very safe with only rare

side effects. The most commonly reported side effects for ciprofloxacin are gastrointestinal symptoms (nausea, vomiting, and diarrhea) in as many as 5 persons in 100. Other reported symptoms in less than 1 person in 100 include rash, dizziness, and headache. Rarely, allergic reactions to these medications have been observed. Ciprofloxacin is not recommended for use in pregnancy due to concerns of joint damage to the unborn child (based on studies in young animals). Pregnancy is exclusionary for study participation and is documented through testing prior to study interventions and provided discussion on methods to prevent pregnancy during study. Fluoroquinolones, including ciprofloxacin, are associated with an increased risk of tendonitis and tendon rupture in all ages. The risk of developing fluoroquinolone-associated tendonitis and tendon rupture is further increased in older patients, usually over 60 years of age, in patients taking corticosteroid drugs, and in patients with kidney, heart, or lung transplants, all of whom are excluded from this study. *Clostridium difficile*-associated diarrhea (CDAD/ pseudomembranous colitis) has been reported with use of nearly all antibacterial agents.

The most common adverse effects of trimethoprim-sulfamethoxazole are gastrointestinal (nausea, vomiting, anorexia), and allergic skin reactions (rash and urticaria). Rare effects can be severe allergic reactions, such as Stevens-Johnson syndrome, toxic epidermal necrolysis, hepatitis, and hematologic abnormalities.

The most common adverse reactions (>1%) of ampicillin are diarrhea, vomiting, and nausea, rash.

Risks Associated with Saliva Collection: Risks occasionally associated with saliva collection include discomfort and (theoretically) choking**.**

Risks Associated with Nasal Swab: Risks occasionally associated with nasal swabs may include discomfort with procedure, nasal irritation, or bleeding.

Risks Associated with Venipuncture or IVs: Risks occasionally associated with venipuncture include pain and bruising at the site of venipuncture, bleeding, infection (occasionally), lightheadedness, and syncope (rarely). IVs may also infiltrate and cause swelling and discomfort.

Risks Associated with Wearable Data Collection: Risks may include feeling uncomfortable about wearing a wrist device in public. The wrist band may also cause some minor wrist irritation.

Risks with Isolation**:** Volunteers may feel bored or anxious about not being able to leave the unit or being separated from family, friends, and community while they are on the unit. The unit will be staffed 24/7 with medical and security staff. There will be planned activities to participate in or not. There are big screen TVs throughout the unit, games, and a kitchen and dining area.

Risks Associated with Laboratory Results: There may be physical, psychological, and social risks if participants test positive for hepatitis B, hepatitis C, and/or HIV. Participants testing positive will be counseled and referred for treatment.

Risks Associated with Collection of Stool: There is no risk associated with collecting stool samples; however, risks of using rectal swabs could include mild discomfort, embarrassment, and (very rarely) bleeding or irritation.

Risks Associated with Post-Infectious Irritable Bowel Syndrome (PI-IBS): Recent studies also suggest an increased risk of PI-IBS following bacterial enteritis. 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 [33].

Risk of Breach of Confidentiality: A breach of confidentiality in which private health information is made public is possible. Medical records associated with this protocol are subject to provisions of the Privacy Act of 1974, 5 U.S.C., Section 552A, and AR 340-21. All data and medical information obtained about participants will be considered privileged and held in confidence. Participants will not be identified by name in any published report/presentation of the results. The Sponsor and the FDA may inspect the records of this research as part of their responsibility to oversee research and ensure protection of participants. Study results and data may be published in scientific/medical journals; the identity of individual participants will not be disclosed.

Risk of SARS-CoV-2 Exposure and Risk Mitigation: SARS-CoV-2, the virus that causes COVID-19 infections and disease, is the cause of the current global pandemic that started in 2020. COVID-19 can cause respiratory symptoms and pulmonary disease. Symptoms include fever, increased cough, shortness of breath, trouble breathing, fatigue, chills, body aches, headache, sore throat, congestion/runny nose, loss of taste or smell, nausea, and diarrhea. The virus can lead to more severe illness including pneumonia, respiratory failure, heart problems, liver problems, blood clots, septic shock, and death.

It is the intent of the Center for Immunization Research to mitigate risk and provide a safe environment for participants while they are inpatient on the unit during the study. The CIR will educate and encourage all potential study volunteers to be fully vaccinated with one of the COVID licensed/EUA vaccines prior to enrolling in this study. Volunteers will not be required to be vaccinated against SARS-CoV-2 however vaccinated volunteers will be prioritized for enrollment.

Pandemic restrictions for vaccinated and unvaccinated persons continue to change based on positivity rates and CDC recommendations. The CIR will follow recommendations from the CDC when implementing clinical trials. The CIR will also follow institutional guidelines and policies. Current recommendations include vaccination with one of the licensed/EUA vaccines for all adults.

Unvaccinated volunteers will be educated on the risks of not being vaccinated. They will sign a disclaimer regarding vaccination status and possible exposure to the virus. Unvaccinated volunteers will be strongly encouraged to wear a mask and maintain social distance from others. Depending on the level of COVID illness in the community and current CDC and JHU guidelines, volunteers may be asked to obtain a COVID-19 test 1-5 days prior to admission.

Every attempt will be made to enroll cohorts of exclusively vaccinated or unvaccinated volunteers. All volunteers will be screened for symptoms and tested on arrival to the unit on the day of inpatient admission. Potential volunteers who test positive for SARS-CoV-2 will not be eligible for enrollment.

For staff working on the unit, vaccination will be required.

As CDC recommendations evolve and change, we will continue to update our plan to incorporate updated CDC recommendations. The addition of precautions or reduced precautions may be instituted throughout the study, updated recommendations will be discussed with volunteers and posted in the unit.

3. Risk Mitigation Strategies

Participants will be questioned and examined daily for evidence of infection and diarrhea complications. Vital signs will be recorded at least three times per day. Based on prior studies, infected participants tend to develop illness with incubation periods of approximately 1-3 days. The risk of diarrhea complications will be minimized by a conservative approach to timing of antibiotic administration, well within an interval that has been shown to be efficacious, as well as daily clinical monitoring. Stool output will be closely monitored. All participants will be treated no later than day 5 post-dosing.

Aggressive fluid management will be undertaken to ensure the most common complication, dehydration, does not occur. The procedures to institute early oral and/or intravenous rehydration therapy are detailed above. In addition to rehydration therapy, prospectively defined criteria and procedures to institute early antibiotic therapy are also fully described above. In order to ensure clinical resolution and limit the potential for secondary spread upon discharge, predefined discharge criteria have been established. Participants will be discharged from the inpatient phase of the study when clinical symptoms are resolved or resolving, they have received at least 2 doses of antibiotics, AND they have two consecutive stool cultures negative for *Shigella*.

Systemic or severe gastrointestinal complications rarely occur with *Shigella* infection. The following clinical findings necessitate immediate consideration and management of complicated enteritis:

- Physical examination compatible with an acute abdomen
- Severe GI bleeding (any evidence of GI blood loss other than hemoccult positivity only, with evidence of hemodynamic instability, decrease in hemoglobin, hypovolemia)
- Sepsis (high fever: temperature >102ºF [39ºC], rigors, hemodynamic instability)

Any of these findings require prompt clinical management and discussion with the independent Research Monitor and the Sponsor Medical Officer.

The *Shigella* strain has the potential for risk to both the environment and to the research personnel; however, the risk to the environment in regard to potential transmission outside of the CIR facility is low. The risk to the environment will be reduced by:

- ensuring that all human waste products from inpatients are disinfected with bleach prior to disposal
- ensuring all participants comply with discharge criteria, emphasizing the importance of handwashing for participants and staff
- ensuring proper disposal/cleaning of linen, and cohorting participants in the CIR while shedding
- ensuring participants will not be discharged until they are no longer shedding the challenge strain, as per procedures outlined in the protocol

Participants with prior history of abnormal bowel patterns who might be at higher risk of post-infectious sequelae are excluded. Predefined criteria to assure early treatment as appropriate also may further reduce risk of post-infectious sequelae and is likely to reduce the risk associated with PI-IBS given the positive association between diarrheal illness duration and PI-IBS risk [34].

It is important for this study that volunteers return for the second challenge if they are invited to do so. We will work with each volunteer to stress this importance, make sure that they are available for both challenges, and address any questions or concerns that they have. It is also important for participants to return for the outpatient visits, and this will be stressed repeatedly with volunteers. We also have some flexibility if volunteers need to alter the dates of outpatient visits.

4. Benefits

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

XX. INFORMED CONSENT

The informed consent process and document(s) will be reviewed and approved by the JHSPH IRB prior to initiation of the study. The consent document(s) will contain a full explanation of the possible risks, advantages, and alternate treatment options, and availability of treatment in the case of injury, in accordance with 21 CFR 50. Participants will receive an oral presentation of the study in language (i.e., using lay terms as appropriate) they can understand. Questions on the purpose of the protocol, protocol procedures, and risks to the participants will then be solicited. Any question that cannot be answered will be referred to the PI. The participant will be allowed to take the consent document home to consider and discuss it with others. The participant should understand that the study products are investigational and are not licensed by the FDA for commercial use but are permitted to be used in this clinical research. Informed consent includes the principle that it is critical the participant be informed about the principal potential risks and benefits. This information will allow the participant to make a personal risk versus benefit decision and understand the following:

- Participation is entirely voluntary.
- Participants may withdraw from participation at any time.
- Refusal to participate involves no penalty.
- The individual is free to ask any questions that will allow him/her to understand the nature of the protocol.

Participants will be given the written, IRB-approved informed consent, allowed ample time to read the consent, allowed to ask questions about the study, have the questions answered, and given time to decide if he/she would like to participate in the study. To document participants' understanding of informed consent, immediately before the consent is signed, the person obtaining consent will administer a brief comprehension test. A participant must achieve ≥70% correct to be eligible for inclusion in the study. Incorrect answers will be discussed with participants to reinforce the consent. Participants who fail the comprehension test on the first attempt will be given one additional opportunity, either on the same day or another day, to take the test after reviewing the quiz, and re-reading the consent. Participants failing the comprehension test on the second attempt are not eligible for study enrollment. No coercion or influence is allowed in obtaining participants' consent.

A written informed consent document, in compliance with 21 CFR Part 50, 32 CFR Part 219, the Belmont Principles will be signed and dated by participants as well as by the PI or designee before any study-related procedures are initiated. This consent document must be retained by the investigator as part of the study records. Participants will receive copies of the signed consent prior to participation. As part of the consent process, participants will also be asked to read and sign a Medical Records/Lab Results Release, with an opportunity to ask questions, if relevant. Participants will also be asked to sign a separate information form for HIV-1 testing. The consent document indicates that by signature, the participant, or where appropriate, legal guardian, permits access to relevant medical records by the Sponsor's representative and by representatives of the FDA.

The Sponsor's representative will submit a copy of the initial IRB- and Sponsor's representative-approved consent form to the FDA and will maintain copies of revised consent documents that have been reviewed and approved by the IRB/ethics committee.

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

All non-exempt research involving human participants shall, at a minimum, meet the requirement of 32 CFR 219.116(a)(6) in the Code of Federal Regulations.

1. Safeguards for Vulnerable Participants:

This study will not include individuals less than 18 years of age, incarcerated, or unable to meet the requirements to sign the informed consent form.

2. Recruitment

A multimedia approach to recruitment will be taken, including the use of social media. Additionally, participants in previous studies that have expressed interest in participating in future trials will be contacted about the proposed study. All study-related advertisements will be reviewed and approved by the JHSPH IRB, NMRC IRB and Human Research Protections Office -Office of Research Protection, if applicable. Participants responding to the advertisements by a phone call to the center will be screened for eligibility based on a standard screening questionnaire administered by the CIR recruiter. Some elements of the inclusion/exclusion criteria will be discussed with the participant at that time and a preliminary determination will be made regarding the individual's eligibility for study participation. Active duty military members will not specifically be recruited for this study.

XXI. COMPENSATION

1. Compensation for Participation

Compensation will occur as detailed below. Compensation will be provided only for completed study procedures designated for compensatory payment. If a participant is eligible to participate in the investigational protocol after screening, and s/he completes all study visits, procedures and follows all the rules s/he will receive the following compensation:

For participants who complete one inpatient phase:

- $100 total for screening (only if enrolled in the study or presents as an alternate)
- $3,150 for each inpatient period (as long as all study requirements are met)
- $300 for each of the outpatient study visits
- $200 bonus for those who complete only 1 challenge including outpatient visits, and
- $300 bonus for those who complete 2 challenges including outpatient visits

If a participant completes the study according to the protocol and does not stay additional days on the inpatient unit, total compensation is $4,950 for participation in one challenge, and $9,100 for participation in 2 challenges.

Volunteers that present to the unit as an alternate but are not admitted will receive $100 for screening (if not already) and $200 for presenting to the unit (a total of $300). Volunteers who are admitted to the unit either for the first or second challenge and are discharged prior to challenge will receive $100 for screening (if not already received) and $250 for each full day on the inpatient unit.

If a participant is not eligible for discharge on day 9 because of illness or not having 2 consecutive negative stool culture results s/he will receive $350 per additional inpatient day.

Participants will not be paid for missed outpatient visits and may forfeit some or all of their bonus as a result of missed/late visits or non-compliance.

2. Compensation for Investigators

There is no financial compensation for investigators in this study. All investigators will be required to complete a form for the disclosure of significant financial interest.

3. Fair and Equitable Selection of Participants

Participants will not be discriminated against on the basis of race, sex, or religion. Due to the nature of the study, we have excluded individuals under 18 and women who are pregnant or nursing and we have excluded individuals who are over the age of 50 due to the frequency of exclusionary medical conditions. Any individual who is unable to consent due to any reason will not be included in this study.

XXII. PRIVACY AND CONFIDENTIALITY

1. Storage of Data and Samples

All records pertaining to this protocol will be stored in a locked filing cabinet at JHU or at an offsite, locked storage facility per regulations for a minimum of 5 years. Access to these records will be limited to researchers in the Translational and Clinical Research Department at NMRC, the JHU CIR and the Sponsor as well as those responsible for regulatory monitoring of data to include representatives of the Sponsor, DoD and JHU. The investigator will obtain permission from the Sponsor in writing before destroying any study records and the Sponsor will notify the investigator in writing when records can be destroyed. Relevant IRBs will be notified in writing prior to destruction of any research records. Specimens will be stored indefinitely in the JHU or the *Shigella* laboratory at WRAIR.

2. Provisions Protecting Privacy and Confidentiality

Individual participant medical information obtained as a result of this study is considered confidential and disclosure to third parties, other than those cited below, is prohibited. Participant confidentiality will be further ensured by utilizing participant identification code numbers and participant initials. Neither the Sponsor, NMRC nor the JHSPH are HIPAA-covered entities.

Confidentiality agreements may be developed with other clinical trials groups (e.g., at the University of Maryland Vaccine Research Center or Walter Reed Clinical Trials Center), and the investigative team may check verbally with these sites to see if participants have participated in studies that would preclude their participation in this study. No written list will be exchanged with these sites.

XXIII. PROTOCOL REVIEW PROCESS

The protocol will undergo scientific review at PATH and NMRC, and ethical review at the CIR. In addition to these reviews, the JHU Biosafety Committee and Pharmacy and Therapeutics Committee will review the protocol. The protocol will also require FDA review as part of the IND application. The IND Sponsor will be PATH. Continuing review will be undertaken in accordance with existing regulations.

XXIV. PUBLICATION POLICY

Results of this study will be presented and published after review by the Sponsor in an open-access

journal as per the guidelines of PATH and the Bill and Melinda Gates Foundation.

XXV. CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with JHU has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

XXVI. REFERENCES

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Table 9: Schedule of Procedures for Subjects Receiving One Challenge

Study Phase	**Outpatient**		**Inpatient**										**Outpatient**				
Study Day	**Screening**	**Screening**	**-2 to -1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**15**	**29**	**57**	**90**	**180**
Compliance Range (Days)	**Day -60 to Day -2**	**Day -30 to Day -2**	-	-	-	-	-	-	-	-	-	-	**±2**	**±4**	**±4**	**-7/ +28**	**±28**
Review Inclusion /Exclusion Criteria	X		X	X													
JH 200 screening consent	X																
Study briefing/Informed Consent	(X)	X															
Comprehension assessment	(X)	X															
HIV testing information sheet	(X)	X															
Functional Bowel Survey		X															X
Medical History	X	X															
Physical examination/Focused PE	(X)	X	X	X	X	X	X	X	X	X	X	X	(X)	(X)	(X)	(X)	(X)
Vital Signs (BP, HR, Temp)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Screening Titers to *S. flexneri* and *S. sonnei* IgG levels	X[3]																
Hematology/CBC w/diff		X	X[1]										X				
Basic Metabolic panel, ALT, AST		X	X[1]										X				
Urine for toxicology testing	X	(X)															
Hepatitis B, C, and HIV		X															
Serum/Urine Pregnancy Test	(X)	X	X	(X)										X			
IgA	X	(X)															
HLA-B27 antigen/ABO typing	X																
COVID-19 testing		X[2]	X														
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Clinical Check/Medical Interview	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Collection of AEs[4]			X	X	X	X	X	X	X	X	X	X	X	X			

Study Phase	Outpatient		Inpatient										Outpatient				
Study Day	**Screening**	**Screening**	**-2 to -1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**15**	**29**	**57**	**90**	**180**
Compliance Range (Days)	**Day -60 to Day -2**	**Day -30 to Day -2**	-	-	-	-	-	-	-	-	-	-	**±2**	**±4**	**±4**	**-7/ +28**	**±28**
Collections of SAEs or AESIs				X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serology including bactericidal antibodies		X	X								X		X	X	X	X	X
Challenge				X													
Stool weighing, grading, assessed for blood				X	X	X	X	X	X	X	X	X					
Stool culture for *Shigella*			X	(X)	X	X	X	X	X	X	X	X					
Stool for IgA			X	(X)			X				X		X	X			
Stool for PCR (RNA later)			X	(X)	X	X	X	X	X	X	X	X					
Stool for Microbiome (RNA Later)			X	(X)	X	X	X	X	X	X	X	X	X	X	X		
Stool for Inflammatory Markers			X	(X)	X	X	X	X	X	X	X	X					
Stool for transcriptomics (RNA later)			X	(X)	X	X	X	X	X	X	X	X	X				
Blood for transcriptomics (systems biology) (whole blood)	(X)	X	X	(X)	X					X			X	X			
Blood for Antigen Arrays (ADI)			X	(X)	X		X			X	X		X	X			X
Antibody lymphocyte supernatant (ALS)		X					X			X	X						
Memory B-Cell			X											X	X	X	X
Serum cytokines			X	(X)	X		X			X							
Cytokines stool			X	X	X	X	X	X	X	X	X	X	X	X			
Salivary IgA			X		X		X		X		X		X	X	X		
Nasal swab for metagenomics			X		X			X			X		X	X	X	X	X
Start antibiotic therapy									X								
Planned discharge												X					
Study completion (1 challenge only)																	X
Post-study safety assessment																	X
Approximate blood volume (mL)#	5	119	63		12.5		74		76.5		84		31	54.5	42	42	52

(X) event may occur on this day in place of another day.

[1]Day -2 to -1 labs are for baseline labs not for determination of eligibility, Day -2 to -1 labs will be obtained on admission to the inpatient unit. Subjects who are completing D90 visit and not being admitted to the inpatient unit will not have safety bloods obtained.
[2]Volunteers will have a COVID-19 test (may be done at local testing site or home) 1 – 5 days prior to admission and will have a COVID-19 test on admission day. If at home antigen-based COVID-19 testing is done, volunteers should be in compliance with current CDC guidelines which include a repeat antigen test 48 hours after a negative first test.
[3]Screening titers may be drawn within 90 days of enrollment.
[4]During the inpatient phase, both solicited and unsolicited adverse events will be assessed, whereas at outpatient visits only unsolicited AEs will be assessed.
Blood volumes are approximate and may change somewhat from these numbers; the total volume will not exceed 550 mL in any 8-week period.

Table 10: Schedule of Procedures for Subjects Receiving Two Challenges

Study Phase	**Outpatient**		**Inpatient**										**Outpatient**				**Inpatient**										**Outpatient**				
Study Day	**Screening**	**Screening**	**-2 or-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**15**	**29**	**57**	**90[1]**	**-2to -1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**15**	**29/ 118**	**57/ 146**	**90/ 180[1]**	**180/ 270[1]**
Compliance Range (Days)	**Day -60 to Day -2**	**Day -30 to Day -2**	-	-	-	-	-	-	-	-	-	-	**±2**	**±4**	**±4**	**-7/ +28**	-	-	-	-	-	-	-	-	-	-	**±2**	**±4**	**±4**	**-7/ +28**	**±28**
Review Inclusion /Exclusion Criteria	X		X	X													X	X													
JH 200 screening consent	X																														
Study briefing/Informed Consent	(X)	X																													
Comprehension assessment	(X)	X																													
HIV testing information sheet	(X)	X																													
Functional Bowel Survey		X																													X
Medical History	X	X																													
Physical examination/Focused PE	(X)	X	X	X	X	X	X	X	X	X	X	X	(X)	(X)	(X)	(X)	X	X	X	X	X	X	X	X	X	X	(X)	(X)	(X)	(X)	(X)
Vital Signs (BP, HR, Temp)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Screening Titers to *S. flexneri* and *S. sonnei* IgG levels	X[6]																														
Hematology/CBC w/diff		X	X										X				X[3]										X				
Basic Metabolic panel, ALT, AST		X	X										X				X[3]										X				
Urine for toxicology testing	X	(X)																													
Hepatitis B, C, and HIV		X																													

Study Phase	Outpatient		Inpatient										Outpatient				Inpatient										Outpatient				
Study Day	**Screening**	**Screening**	**-2 or-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**15**	**29**	**57**	**90[1]**	**-2to -1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**15**	**29/ 118**	**57/ 146**	**90/ 180[1]**	**180/ 270[1]**
Compliance Range (Days)	**Day -60 to Day -2**	**Day -30 to Day -2**	-	-	-	-	-	-	-	-	-	-	±2	±4	±4	-7/ +28	-	-	-	-	-	-	-	-	-	-	±2	±4	±4	-7/ +28	±28
Serum/Urine Pregnancy Test	(X)	X	X	(X)										X			X	(X)										X			
IgA	X	(X)																													
HLA-B27 antigen/ABO typing	X																														
COVID-19 test		X[4]	X														X														
Concomitant Medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X			
Clinical Check/Medical Interview	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Collection of AEs[2]			X	X	X	X	X	X	X	X	X	X	X	X				X	X	X	X	X	X	X	X	X	X	X			
Collections of SAEs or AESIs				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Serology including bactericidal antibodies		X	X								X		X	X	X	X	X								X		X	X	X	X	X
Challenge				X														X													
Stool weighing, grading, assessed for blood				X	X	X	X	X	X	X	X	X						X	X	X	X	X	X	X	X	X					
Stool culture for *Shigella*			X	(X)	X	X	X	X	X	X	X	X					X	(X)	X	X	X	X	X	X	X	X					
Stool for IgA			X	(X)			X				X		X	X			X	(X)			X				X		X	X			
Stool for PCR (RNA later)			X	(X)	X	X	X	X	X	X	X	X					X	(X)	X	X	X	X	X	X	X	X					
Stool for Microbiome (RNA Later)			X	(X)	X	X	X	X	X	X	X	X	X	X	X		X	(X)	X	X	X	X	X	X	X	X	X	X	X		
Stool for Inflammatory Markers			X	(X)	X	X	X	X	X	X	X	X					X	(X)	X	X	X	X	X	X	X	X					
Stool for transcriptomics (RNA later)			X	(X)	X	X	X	X	X	X	X	X	X				X	(X)	X	X	X	X	X	X	X	X	X				
Blood for transcriptomics (systems biology) (whole blood)	(X)	X	X	(X)	X				X				X	X			X	(X)	X					X			X	X			
Blood for Antigen Arrays (ADI)			X	(X)	X		X		X		X		X	X			X	(X)	X		X			X	X		X	X			X

Study Phase	Outpatient		Inpatient										Outpatient				Inpatient										Outpatient				
Study Day	**Screening**	**Screening**	**-2 or-1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**15**	**29**	**57**	**90[1]**	**-2to -1**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**15**	**29/ 118**	**57/ 146**	**90/ 180[1]**	**180/ 270[1]**
Compliance Range (Days)	**Day -60 to Day -2**	**Day -30 to Day -2**	-	-	-	-	-	-	-	-	-	-	**±2**	**±4**	**±4**	**-7/ +28**	-	-	-	-	-	-	-	-	-	-	**±2**	**±4**	**±4**	**-7/ +28**	**±28**
Antibody lymphocyte supernatant (ALS)		X					X		X		X						X[5]				X			X	X						
Memory B-Cell			X											X	X	X	X											X	X	X	X
Serum cytokines			X	(X)	X		X		X								X	(X)	X		X			X							
Cytokines stool			X	X	X	X	X	X	X	X	X	X	X	X			X	X	X	X	X	X	X	X	X	X	X	X				
Salivary IgA			X		X		X		X		X		X	X	X		X		X		X		X		X		X	X	X		
Nasal swab for metagenomics			X		X			X			X		X	X	X	X	X		X			X			X		X	X	X	X	X
Start antibiotic therapy									X														X								
Planned discharge												X														X					
Study completion (RE-challenge only)																															X
Post-study safety assessment																															X
Approximate Blood volumes (mL)#	5	113.5	63		12.5		74		76.5		84		31	54.5	42	42	63 naïve 127 veterans		12.5		74		76.5		84		31	54.5	42	42	52

[1]Participants who receive the second challenge will have their day 90 and day 180 after the second challenge. If the second challenge is >120 days after the first, the participants will have a day 90 and a day-2 or -1 visit.

[2]During the inpatient phase, both solicited and unsolicited adverse events will be assessed, whereas at outpatient visits only unsolicited AEs will be assessed.

Blood volumes are approximate and may change somewhat from these numbers; the total volume will not exceed 550 mL in any 8-week period.

[3]Second admission, Day -2 or -1 safety labs are for baseline labs not for determination of eligibility, Day -2 or -1 labs will be obtained on admission to the inpatient unit. Subjects who are completing D90 visit and not being admitted to the inpatient unit will not require safety bloods.

[4]Volunteers will have a COVID-19 test (may be done at local testing site or home) 1 – 5 days prior to admission and will have a COVID-19 test on admission day. Volunteers for 2nd admission will have a COVID-19 test (may be done at local testing site or home) 1 – 5 days prior to admission and will have a COVID-19 test on admission day. If at home antigen-based COVID-19 testing is done, volunteers should be in compliance with current guidelines which includes a repeat antigen test 48 hours after a negative first test.

[5]Only subjects returning for second challenge will need ALS drawn on day -2 or -1 second admission (naïve subjects will have pre-study ALS collected during screening).

[6]Screening titers may be drawn within 90 days of enrollment.

(X) Event may occur on this day in place of another day.