Phase 1 Randomized, Placebo-Controlled, Dose-Escalation Study of the Safety, Reactogenicity, and Immunogenicity of Trivalent *Salmonella* (*S.* Enteritidis/*S.* Typhimurium/*S.* Typhi Vi) Conjugate Vaccine against Invasive *Salmonella* Disease Administered Parenterally to Healthy U.S. Adults

Protocol Number: *Salmonella* conjugates CVD 1000

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STATEMENT OF COMPLIANCE

This trial is to be conducted in accordance with Good Clinical Practices (GCP) as required by the following:

- United States Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 54, 21 CFR Part 56, and 21 CFR Part 312)
- International Conference on Harmonization (ICH) E6; 62 Federal Register 25691 (1997)
- The Health Insurance Portability and Accountability Act (HIPAA) Privacy Rule-Final Modification (45 CFR Parts 160 and 164)
- The local laws and regulations, which prevail over the University of Maryland, Baltimore

Compliance with these standards provides public assurance that the rights, safety, and well-being of study subjects are protected, consistent with the principles that have their origin in the Declaration of Helsinki.

All key personnel (all individuals responsible for the design and conduct of this trial) have completed Human Subjects Protection Training.

SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial is to be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable United States of America federal regulations and ICH guidelines.

Principal Investigator: _____
Name / Title (Print)

Signature: _____

Date: _____

Table of Contents

ABBREVIATIONS

ADH	Adipic acid dihydrazide
AE	Adverse Event
ALT	Alanine aminotransferase
ALS	Antibodies in lymphocyte supernatant
ANC	Absolute neutrophil count
ASC	Antibody secreting cells
AST	Aspartate Aminotransferase
B_M	B memory cell
B_n	Naïve B cell
BMI	Body mass index
BP	Blood pressure
BUN	Blood urea nitrogen
CBC	Complete blood count
CDAP	1-cyano-4-dimethylaminopyridinium tetrafluoroborate
CFR	Code of Federal Regulations
CFU	Colony forming units
cGMP	Current Good Manufacturing Practices
CI	Confidence interval
CMI	Cell-mediated immunity
COPS	Core-O polysaccharide
COVID-19	Coronavirus disease 2019, caused by the SARS-CoV-2 virus
CRF	Case report form
CRP	C-reactive protein
cT_{fh}	Circulating T follicular/helper cell
CVD	Center for Vaccine Development
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot
FDA	Food and Drug Administration
FliC	Flagellin subunit protein (phase 1 for *S*. Typhimurium)
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMBS	succinimidyl 4-maleimidylbutyrate (a.k.a. N-γ-maleimidobutyryl-oxysuccinimide ester)
GMT	Geometric mean titer
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C virus
HEENT	Head, eyes, ear, nose, throat
Hg	Hemoglobin
HIPAA	Health Insurance Portability and Accountability Act
HIV	Human Immunodeficiency Virus
HRP	Horseradish peroxidase
HSF1	Health Sciences Facility building 1
ICF	Informed consent form
IDS	Investigational Drug Service

ICH	International Conference on Harmonization
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IM	Intramuscular
IND	Investigational new drug
iNTS	Invasive non-typhoidal *Salmonella*
IP	Investigational product (a.k.a. study product)
IRB	Institutional Review Board
ISM	Independent safety monitor
IU	International units
KDO	2-keto-3-deoxyoctonate
LPS	Lipopolysaccharide
mcg or µg	Micrograms
mL	Milliliters
MOP	Manual of procedures
NTS	Non-typhoidal *Salmonella*
OPA	Opsonophagocytic activity
OPS	O polysaccharide
PBS	Phosphate buffered saline
pH	Potential of hydrogen
PBMC	Peripheral blood mononuclear cells
PI	Principal investigator
QA	Quality assurance
QC	Quality control
QMP	Quality management plan
S. (*italicized S.*)	*Salmonella*, bacteria of the genus
SAE	Serious adverse event
SBA	Serum bactericidal activity
SD	Standard deviation
SFC	Spot-forming cell
SMC	Safety monitoring committee
SOP	Standard operating procedure
TBIL	Total bilirubin
TLR5	Toll-like receptor 5
TT	Tetanus toxoid
U.S. or U.S.A.	United States (of America)
WBC	White blood cell
WHO	World Health Organization

PROTOCOL SYNOPSIS

Title:	**Phase 1 Randomized, Placebo-Controlled, Dose-Escalation Study of the Safety, Reactogenicity, and Immunogenicity of a Trivalent (*S.* Enteritidis/*S.* Typhimurium/*S.* Typhi Vi) Conjugate Vaccine against Invasive *Salmonella* Disease Administered Parenterally to Healthy U.S. Adults**
Phase:	1, adaptive design
Population:	104 healthy adults, ages 18 through 45
Site:	Two site, outpatient trial
Subject Participation Duration:	Each subject will participate for approximately 6-7 months from the time of enrollment through completion of the study.
Investigational Products:	• **Study product #1**: trivalent *S*. Enteritidis [25 µg]/*S*. Typhimurium [25 µg]/*S*. Typhi Vi conjugate vaccine, presented in multi-dose vials containing 2-phenoxyethanol preservative. • **Study product #2**: placebo, a buffer containing the 2-phenoxyethanol preservative.
Primary Objectives:	1. Safety and Reactogenicity: To assess the frequency and severity of solicited local (i.e., injective site) and systemic (such as fever) AEs during the first 7 days following each dose of vaccine. 2. Safety: To assess the frequency and severity of unsolicited AEs within 28 days of each dose of vaccine and the occurrence of any SAEs through 6 months after the last dose of vaccine 3. Immunogenicity: To measure the proportion of subjects that achieve a four-fold increase in titer, as compared to baseline, of specific serum IgG anti-COPS (*S*. Enteritidis or *S*. Typhimurium), anti-Vi (*S*. Typhi) polysaccharide, and anti-FliC (*S*. Enteritidis or *S*. Typhimurium) antibody at days 29 and 57, as measured by ELISA.
Secondary Objectives	1. Safety: To assess the frequency and severity of abnormalities in clinical safety laboratory parameters at 7 days following the first dose of vaccine. 2. Immunogenicity: To measure the proportion of subjects that achieve a four-fold increase in titer, as compared to baseline, of specific serum IgG anti-COPS (*S*. Enteritidis or *S*. Typhimurium), anti-Vi (*S*. Typhi) polysaccharide, and anti-FliC (*S*. Enteritidis or *S*. Typhimurium) antibody at day 180/210, as measured by ELISA. 3. Immunogenicity: To calculate the GMT of serum IgG anti-COPS (*S*. Enteritidis or *S*. Typhimurium), anti-Vi polysaccharide, and anti-FliC (*S*. Enteritidis or *S*. Typhimurium) antibodies at days 1, 29, 57, and 180/210, as measured by ELISA.

Exploratory Objectives

1. Immunogenicity: To measure the titers of functional antibodies by serum bactericidal antibody (SBA) activity assay and opsonophagocytic activity (OPA) assay against *S*. Typhimurium, *S*. Enteritidis, and *S*. Typhi bacterial cells at days 1, 29, 57, and 180/210.
2. Immunogenicity: To measure the antibody secreting cells (ASC), specific for anti-COPS, Vi, FliC, and TT; possibly including homing markers and the measurement of antibodies in lymphocyte supernatants (ALS)
3. Immunogenicity: To measure memory B and T cells, specific for anti-COPS, anti-Vi, anti-FliC, and anti-TT activity; including the B cells that exhibit mucosal homing markers integrin α4β7 and/or the lymph node homing marker CD62L
4. Immunogenicity: To conduct functional genomic and proteomic studies to further explore the immune response to vaccination.
5. Immunogenicity: To measure the anti-Vi polysaccharide, anti-TT, anti-COPS, and anti-FliC antibody levels in oral fluid at days 1, 29, 57, and 18/210, as measured by ELISA (Cohorts C and D)

Brief Description of Study Design

This is intended to be an adaptive design study.[1] In Part 1 of the study, we will conduct a double-blinded, randomized, placebo-controlled, dose-escalation study. Participants will be sequentially enrolled into Part 1 of the study in three dose-escalating steps (Steps 1-3, Cohorts A-C), consisting of fractional vaccine doses containing: 6.25 µg (Step 1), 12.5 µg (Step 2), or 25 µg (Step 3) of each specific polysaccharide by weight. Within each step, participants will be randomly allocated to receive a single dose of trivalent conjugate vaccine or placebo. In Step 3, participants may receive one dose or two-doses, separated by 28 days, of the vaccine.

Part 2 of the study is intended to expand the safety and immunogenicity data from a selected dose level of the vaccine (anticipated to be the 25 µg per conjugate dose level). The target dose for this part of the study is to be informed by safety and immunogenicity data from the dose-escalation cohorts, with an intent to select the most promising immunogenic dose of the vaccine. Part 2 of the study will consist of a cohort of 60 participants who will be randomly allocated to receive a blinded two-dose regimen in one of the following three possible treatment groups:

- a single dose of the trivalent conjugate vaccine, followed by a dose of placebo 28 days later (n=25);
- two doses of trivalent conjugate vaccine, separated by 28 days (n=25);
- or two doses of placebo, separated by 28 days (n=10).

Special Notation: Due to the limited shelf-life of the vaccine, which is being determined by an ongoing stability testing plan, the availability of this pilot production lot of vaccine may be limited in time. Furthermore, since March 2020 the global pandemic due to SARS-CoV-2 virus (causing COVID-19 infections) has significantly disrupted the ability to conduct this study. Therefore, the Step 3 (Cohort C) has been significantly revised such that both a single and two-dose regimens of the 25 µg dosage of vaccine will be evaluated, albeit with a very small sample size. This was originally intended to be evaluated with a large sample size in Part 2 (Cohort D), but due to the extraordinary present circumstances, the revised design of the study is intended to provide similar information which is crucial for the vaccine development plan. Furthermore, in order to provide some bridging immunogenicity data, a small number of participants in Cohort C will be randomized to receive either a single or two-dose regimen of

the 12.5 µg dosage of the vaccine. It is possible that Part 2 (Cohort D) will not be conducted, due to lack of vaccine.

Furthermore, because of the COVID-19 situation, for Cohort A the follow-up visits at 6 months post-vaccination were conducted by phone call contact and thus the collection of specimens was unable to be conducted. For Cohort B, the follow-up visits at 2 months and 6 months post-vaccination were conducted by phone call contact and thus the collection of specimens was unable to be conducted. Therefore for Cohort A and B participants, a single clinic visit for the collection of blood and oral fluid will be requested to measure the long-term immune responses (>6 months post-vaccination).

Pharmaron CPC, Inc. (Baltimore, MD) is being added as a site for the enrollment of Cohort C.

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1 BACKGROUND

1.1 Epidemiology and Burden of Disease

Salmonella enterica serovars Typhi and Paratyphi A and B and certain non-typhoidal *Salmonella enterica* (NTS) serovars are important causes of invasive *Salmonella* disease worldwide. "Typhoid Fever" is caused by *Salmonella enterica* serovar Typhi (*S*. Typhi), while "Paratyphoid Fever" is caused by *Salmonella enterica* serovar Paratyphi A (*S*. Paratyphi A) or *Salmonella enterica* serovar Paratyphi B (*S*. Paratyphi B). Both Typhoid Fever and Paratyphoid Fever manifest as persistent fevers, abdominal discomfort, headache and malaise. Without prompt and effective antibiotic treatment, these infections may lead to intestinal hemorrhage, intestinal perforation and peritonitis, myocarditis, hepatitis, neurological consequences and death. In the pre-antibiotic era the case fatality rate of typhoid fever was circa 15%.

In industrialized countries in North America, Europe and in Australia and New Zealand, various NTS serovars are common causes of gastroenteritis, as the result of sporadic food-borne transmission or associated with outbreaks. In older children and adults, these NTS infections typically result in uncomplicated diarrheal illness that does not require antibiotic treatment and invasive infections due to NTS serovars in healthy adults are rare. However, among certain sub-populations (such as infants < 3 months of age, the elderly, and immunocompromised hosts) in industrialized countries, invasive NTS (iNTS) infections such as bacteremia, septicemia, and meningitis can be observed and are associated with notable case fatality.[2,3] The most common *Salmonella* serovars associated with invasive disease in the U.S. include: *S*. Typhimurium, *S*. Enteritidis, *S*. Heidelberg, *S*. Dublin, and *S*. Schwarzengrund.[4]

Whereas invasive disease due to NTS (bacteremia, septicemia, meningitis, etc.) is relatively rare in the USA and industrialized countries, a recognized burden of iNTS disease exists in sub-Saharan Africa, where it was recently estimated that ~2 million cases (~227 cases/100,000 infants and young children) occur annually.[5] The case fatality ratio of iNTS disease in Africa is ~20%, resulting in an estimated ~681,000 deaths each year.[5] In sub-Saharan Africa four serovars, *S*. Typhimurium, *S*. Enteritidis, I:4,[5],12:i-, and *S*. Dublin, account for > 85-95% of all iNTS cases.[6,7] Notably, the strains of *S*. Typhimurium, I:4,[5],12:i- and *S*. Enteritidis associated with invasive NTS disease in sub-Saharan Africa are genomically distinct from strains of those serovars circulating in the USA and Europe.[8,9] For example, *S*. Typhimurium and I:4,[5],12:i- strains in the USA and Europe are overwhelmingly of multi-locus sequence type ST19, whereas the African strains are ST313, an unusual MLST type. Similarly, the *S*. Enteritidis strains in Africa are genomically distinct. Notably, most cases of iNTS in infants and toddlers in sub-Saharan Africa do not present with or have a history of gastroenteritis. The vast majority of the iNTS isolates from sub-Saharan Africa encode resistance to multiple clinically-relevant antibiotics.[8,10]

1.2 Vaccine Development

To address this important public health problem, iNTS vaccines are being developed.[11,12] The polysaccharides of encapsulated bacteria (e.g., *Haemophilus influenzae* type b, *Streptococcus pneumoniae*, *Neisseria meningitidis*) linked to carrier proteins have resulted in safe, immunogenic (in young children) and highly effective parenteral polysaccharide conjugate

vaccines. One typhoid polysaccharide conjugate vaccine, Typbar-TCV®, manufactured by Bharat Biotech, Hyderabad, India, that was first licensed in India, was pre-qualified in 2017 by the World Health Organization (WHO) for procurement by United Nations Agencies and its use in infants as young as six months of age has been recommended by WHO's Scientific Group of Experts (SAGE).[13,14] In contrast, currently there are no licensed NTS vaccines.

CVD investigators have been developing candidate polysaccharide protein conjugate vaccines for the prevention of iNTS disease. The vaccine development strategy involves targeting two surface virulence antigens of the key NTS *Salmonella* serovars including the core polysaccharide and O-polysaccharide (COPS) plus FliC protein, the unpolymerized flagellin subunits of flagella motility organelles.[12,15-19]

The outer membrane lipopolysaccharides of *Salmonella* can be divided into 3 distinct regions: an endotoxin lipid A moiety; a highly conserved, core oligosaccharide region; and a serogroup-specific, repeating oligosaccharide polymer (O-polysaccharide [OPS]). The majority of human invasive isolates fall antigenically within *Salmonella* group A (includes *S*. Paratyphi A), Group B (includes *S*. Paratyphi B, *S*. Typhimurium and I:4,[5],12:i-), Group C (includes *S*. Paratyphi C, *S*. Choleraesuis and *S*. Newport), and Group D (includes *S*. Typhi, *S*. Enteritidis and *S*. Dublin). Serovars of *Salmonella* serogroups A, B, and D share a common mannose-rhamnose-galactose trisaccharide backbone. Distinct dideoxyhexose sugars linked to the mannose residues of the common side chain provide serogroup specificity. Thus, if the dideoxyhexose sugar linked to the mannose is a paratose, this results in immunodominant antigen "2", denoting a serogroup A *Salmonella* isolate. If the sugar linked to the mannose is an abequose, this results in an immunodominant antigen "4", denoting serogroup B *Salmonella*. Finally, if the sugar linked to the mannose is a tyvelose, this results in immunodominant antigen "9", denoting serogroup D *Salmonella*.[12]

Specific antibody responses against the O polysaccharide antigens of the conjugate, if protective, should provide some degree of protection against all the different serovar pathogens within that O serogroup. For example, both *S*. Enteritidis and *S*. Dublin serovars fall within O serogroup D. Immune responses against Group D O antigen should contribute to protection against both of these Group D serovars. CVD investigators have devised practical methods to purify COPS from attenuated "reagent strains" that have been engineered to enhance the yield of COPS and to facilitate the large-scale manufacture of these antigens under enhanced occupational safety.[18] The vaccine candidates to be evaluated in this study are based on the COPS of *S*. Typhimurium (a Group B serovar) and *S*. Enteritidis (a Group D serovar).

The *S*. Enteritidis conjugate component in our trivalent vaccine is generated by 1-cyano-4-dimethylaminopyridinium tetrafluoroborate (CDAP) chemistry to link *S*. Enteritidis COPS to the FliC Phase 1 flagellin protein subunits of *S*. Enteritidis that had been derivatized with adipic acid dihydrazide (ADH) using carbodiimide. This strategy is similar to the approach used for MenAfriVac™, which is comprised of the group A meningococcus capsular polysaccharide linked to tetanus toxoid (TT).[20] MenAfriVac has been administered to infants, toddlers, children, and adults in countries within the African Meningitis belt.[21] CDAP linkage and ADH derivitization are also used by GlaxoSmithKline (GSK) Vaccines in preparing their multivalent pneumococcal conjugate vaccine (Synflorix™) and by Sanofi Pasteur Vaccines in preparing their quadrivalent meningococcal conjugate vaccine (Menactra™). Synflorix™ and Menactra™ have extensive track records of safety in infants, children and adults, including in sub-Saharan Africa. Similarly, experimental *S*. Paratyphi A conjugates generated at the National Institute of Child Health and

Human Development by John B. Robbins and colleagues in the late 1990s consisted of *S*. Paratyphi A COPS linked to tetanus toxoid carrier protein and utilized ADH as a linker with CDAP conjugation.[22] The *S*. Paratyphi A conjugates prepared by the Robbins Laboratory at NICHD were safe and immunogenic in Phase 1 and Phase 2 clinical trials that involved adults, teenagers and children 2-4 years of age.[23] Interestingly, among the pre-school age children who received either one or two spaced doses of *S*. Paratyphi A conjugate 6 weeks apart, there was no evidence of a booster effect following administration of the second dose of conjugate.[23]

The *S*. Typhimurium conjugate component in our trivalent vaccine is generated by modification at the polysaccharide terminal 2-keto-3-deoxyoctonate (KDO) carbonyl with an aminooxythiol linker. The aminooxy forms an oxime bond with the KDO ketone that is further reduced with sodium cyanoborohydride. *S*. Typhimurium FliC protein (phase 1 flagellin subunit) is derivatized with the amine reactive reagent succinimidyl 4-maleimidylbutyrate (GMBS) to introduce a maleimide moiety that is then linked to the reactive sulfhydryl of the derivatized COPS molecule via formation of a thioether bond. There is no FDA-licensed conjugate vaccine for human use that utilizes the specific method of conjugation employed for our *S*. Typhimurium glycoconjugate. However, a synthetic Hib capsular polysaccharide-based glyconjugate vaccine that is licensed and manufactured in Cuba (and that is pre-qualified by the WHO) utilizes a maleimide linker.[24] We have extensive pre-clinical experience with *S*. Typhimurium conjugate vaccines prepared utilizing this approach, and have published reports that document the tolerability and immunogenicity of such conjugates in animal models.[15,16,19,25] John Robbins and team at the NICHD also successfully used aminooxy linker based approaches for synthesis and preclinical evaluation of COPS-based conjugates for a variety of gram-negative pathogens.[26,27]

The *S*. Typhi conjugate component in our trivalent conjugate vaccine is essentially Typbar-TCV™, a Vi conjugate vaccine generated by carbodiimide-mediated modification of *S*. Typhi Vi polysaccharide with ADH that introduces reactive hydrazide groups that are then used to link to tetanus toxoid (TT) via a second carbodiimide step. Typbar-TCV is a licensed typhoid vaccine in India and a number of other countries and has been extensively used in pediatric and adult populations.[28] The World Health Organization conferred pre-qualification status for Typbar-TCV on January 3, 2018.[14,29] It is now a vaccine which can be procured by United Nations agencies.

Therefore, CVD investigators have devised novel candidate core-O polysaccharide-flagellin protein conjugates as a vaccine strategy for preventing iNTS and we have combined the iNTS conjugates with a Vi-TT conjugate (Typbar-TCV) to create a Trivalent Conjugate vaccine for preventing invasive iNTS disease and typhoid fever.

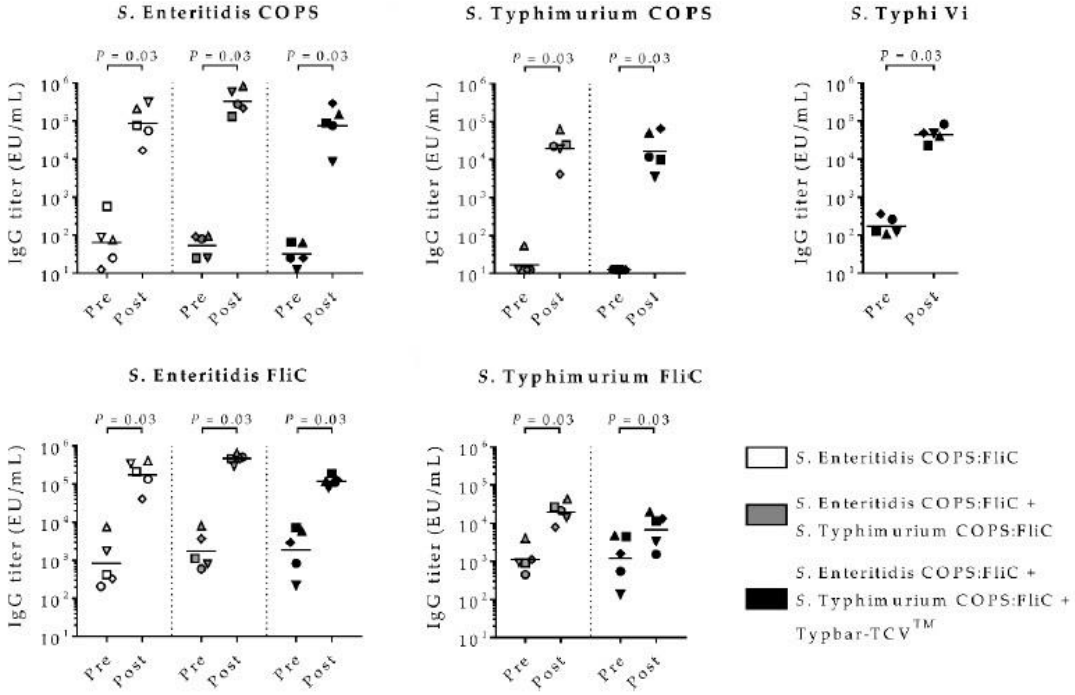
1.3 Pre-clinical Testing

Early non-GMP formulations of monovalent *S*. Enteritidis and *S*. Typhimurium COPS:FliC conjugate vaccines were immunogenic and protected mice against fatal infections with the homologous serovar NTS pathogen.[15-19]

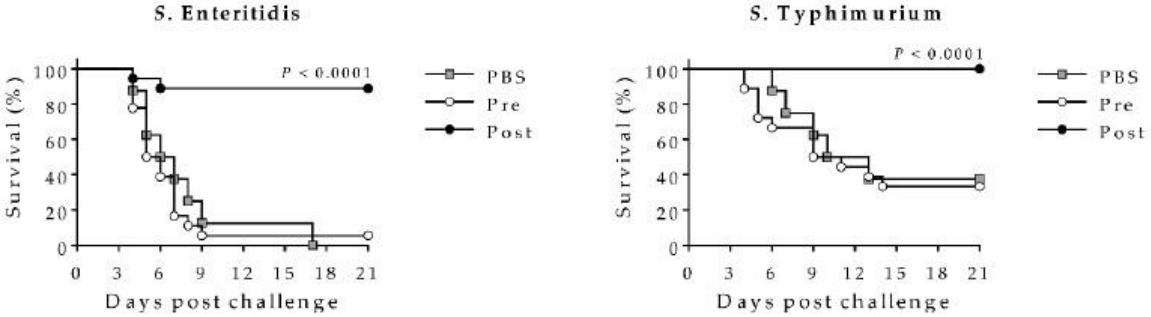
1.3.1 Rabbit non-GMP product study

Non-GMP bivalent and trivalent formulations of the *Salmonella* conjugate vaccines were also evaluated in a rabbit model.[15] New Zealand White rabbits were immunized with 3 sequential doses of a monovalent *S*. Enteritidis, bivalent *S*. Enteritidis/Typhimurium, and trivalent *S*. Enteritidis/Typhimurium/Typhi conjugate vaccine formulations, separated 14 days apart, and

serum was collected pre-vaccination and post-final immunization (at day 44). High titers of serogroup-specific anti-COPS and anti-Vi (in the trivalent formulation) IgG antibodies were elicited by the bivalent and trivalent formulations; robust anti-FliC antibody were also elicited but the responses to FliC were lower relative to the anti-COPS titers. (Figure below)



In vitro functional antibody activity was demonstrated with the complement-mediated serum bactericidal activity (SBA) assay. Furthermore, *in vivo* functional antibody activity was measured with outbred CD-1 mice which were passively immunized with pooled pre- or post-vaccination rabbit sera or PBS; then subsequently challenged intraperitoneally with *S*. Enteritidis R11 or *S*. Typhimurium D65 (representative virulent Malian blood serovar isolates). Mice which received PBS or pre-vaccination sera demonstrated >90% fatality with *S*. Enteritidis and ~63% fatality with *S*. Typhimurium, whereas mice that received post-vaccination serum were protected against lethal challenge (vaccine efficacy ~88% for *S*. Enteritidis and 100% for *S*. Typhimurium). The Kaplan-Meier survival curves for the respective pre- and post-immune sera were compared using log-rank analysis and P values were calculated as <0.0001 for both serovar challenges.[15] (Figure below)



1.3.2 GLP Toxicology Study

As part of our submission of an Investigational New Drug Application (IND) to the U.S. Food and Drug Administration (FDA), the four products manufactured at Bharat Biotech International, Inc. and two of which are to be tested in the Phase 1 trial described herein have been evaluated in a Rabbit Toxicology Test performed by Noble Life Sciences, a Contract Research Organization located in Sykesville, Carrol County, MD, that specializes in performing animal toxicology tests under the tenets of Good Laboratory Practices (GLP). Eighty pathogen-free rabbits, 40 males and 40 females, were injected; 20 rabbits with each vaccine test product (3 vaccine test products: single-dose bivalent, multi-dose bivalent, and multi-dose trivalent) and 20 with the placebo test product (buffer containing 2-phenoxyethanol). The Rabbit Toxicology study was initiated July 20, 2018 and the first dose of study vaccines were administered on August 13, 2018; a total of four sequential doses separated by 28 days was administered. All animals survived to the scheduled necropsy. There were no test article-related adverse changes in mortality, clinical observations, physical exams, injection site scores, body weights, food consumption, organ weights, organ-to-body weight ratios, body temperature, ophthalmology or urinalysis. The antigen-specific ELISA IgG responses following the first dose of vaccine for the 20 rabbits that received the trivalent multi-dose test article are shown in the table below. According to these pre-clinical data, the vaccines appear to be immunogenic after a single dose.

ELISA IgG response following the first dose of vaccine for rabbits that received the trivalent multi-dose vaccine

Typhimurium titers (EU/mL)		Enteritidis titers (EU/mL)		Typhi Vi titers (EU/mL)	
GMT, pre	3.4	GMT, pre	7.2	GMT, pre	24.0
GMT, post (day 29)	1454.2	GMT, post (day 29)	4381.4	GMT, post (day 29)	2409.5
Geometric Mean Fold-rise	426.8	Geometric Mean Fold-rise	610.6	Geometric Mean Fold-rise	99.7
Minimum fold-rise	41.0	Minimum fold-rise	75.6	Minimum fold-rise	12.5
Maximum fold-rise	6977.1	Maximum fold-rise	3202.8	Maximum fold-rise	337.5

1.4 Clinical Studies

No prior human clinical studies have been performed with the trivalent (iNTS conjugates combined with Typhoid Vi conjugate) candidate *Salmonella* vaccines, that will be evaluated in the study described in this clinical protocol.

However, the *S*. Typhi Vi capsular polysaccharide-TT conjugate vaccine (Typbar-TCV®, Bharat Biotech, Hyderabad, India) component is licensed as a monovalent conjugate vaccine in India[28] and has been pre-qualified by the WHO and recommended by the WHO SAGE for use in adults, children and infants as young as 6 months of age. Among the adverse effects associated with Typbar-TCV are the following:[30]

- Common (≥1% and <10%): fever, pain at injection site, and swelling
- Uncommmon (≥0.1% and <1%): injection site tenderness, itching, arthralgia, cold, cough, vomiting, and myalgia

The iNTS conjugates in our trivalent vaccine use Phase 1 flagellin subunits from the homologous iNTS serovars as the carrier protein. Flagellin can act as an adjuvant through binding to Toll-like receptor 5 (TLR5) and the induction of innate and adaptive immune signaling via an MyD88-dependent pathway and an MyD88-independent mechanism.[31,32] Genetically-engineered fusion proteins consisting of influenza virus hemagglutinin or matrix protein fused to a TLR5 stimulating portion of the FljB flagellin subunit of *S*. Typhimurium Phase 2 flagella have been evaluated in clinical trials. Some dose levels were associated with reactogenicity and

elevated levels of C-reactive protein, suggesting strong stimulation of the innate immune system.[33-35] Importantly, the method of linkage of the Phase 1 flagellin FliC subunits to COPS in our NTS conjugates masks the TLR5 stimulating portion of flagellin. Pre-clinical assays of our conjugates have demonstrated >100-fold reduction in TLR5 activity using an NF-κB-luciferase reporter assay. Most importantly, rabbits tolerated the vaccine without mounting febrile responses or exhibiting elevated C-reactive protein levels following vaccination in the rabbit toxicology test.

There is considerable justification for our expectation that a 25 µg (by weight of polysaccharide per dose of each conjugate) dose of our iNTS conjugate vaccine will be safe and immunogenic, based on results with various Vi conjugate vaccines containing 25 µg of Vi linked to various carrier proteins. As mentioned, Typbar-TCV, which contains 25 µg of Vi polysaccharide linked to TT, has an excellent safety record in adults, children and infants.[28]

In a field trial in Vietnam involving children 2-5 years of age, a two-dose regimen of another Vi conjugate vaccine consisting of 25 µg of *S*. Typhi Vi polysaccharide linked to recombinant exotoxin A of *Pseudomonas aeruginosa* as the carrier protein was well tolerated, immunogenic and significantly protective against confirmed typhoid fever.[36]

1.5 Risks and Potential Benefits

1.5.1 Risks

Risks related to Bivalent or Trivalent vaccine:

Since this is an investigational vaccine and has not been given to humans previously, the risks associated with administration are unknown. These vaccines are killed and purified and will not cause *Salmonella* infection. With an injectable vaccine, recipients may have fever, swelling, redness and pain at the injection site. An allergic reaction, including anaphylaxis, could possibly occur. The Typhoid component of the trivalent vaccine is virtually the same as the licensed vaccine called Typbar-TCV™. This licensed vaccine is known to cause fever in 10% of children who receive it.

Blood Drawing risks:

Blood drawing is sometimes associated with pain at the site from which the blood is drawn, bruising, and occasional lightheadedness and rarely, fainting or infection. Bruising at the site of blood drawing can be prevented by applying pressure for several minutes. To reduce the risk of infection, the area is wiped clean with alcohol and germ-free equipment is used. It is the policy of the American Red Cross that recipients of an experimental vaccine may not be able to donate blood for one year after receipt.

Risks of Confidentiality:

Efforts will be made to keep personal health information confidential. There is a small risk of loss of confidentiality by an unauthorized person viewing study participant records. In order to maintain confidentiality, the PI and the study staff will store study records in a locked area of a limited access locked office. Study records and samples will be coded with a number, not the participant's name. Records will be used by the FDA, the data coordinating center and monitoring group, CVD and the IRB only in connection with carrying out their obligations relating to the study, and every effort will be made to keep the records as confidential as possible, within the limits of the law.

Risks to Pregnancy or Fetus:

If a woman is pregnant or becomes pregnant, this research may hurt the baby or the pregnancy in ways that are unknown. Women of childbearing potential must use birth control to be sure that they do not become pregnant. The only birth control methods that work well enough to be sure that women of childbearing potential will not become pregnant are oral contraceptives ("the pill"), intrauterine devices (IUDs), contraceptive implants under the skin, or contraceptive injections, and condoms with foam.

1.5.2 Potential Benefits

Participants will not benefit directly from their participation in this study. However, their participation may help the investigators better understand the response to these *Salmonella* vaccines and whether these vaccines will help to prevent the disease.

2 STUDY DESIGN

Part 1. The first part of the study will be conducted as a traditional first-in-human, double-blinded, randomized, placebo-controlled, dose-escalation study. This part of the study is designed to assess the safety, reactogenicity, and preliminary immunogenicity of a single parenteral (i.e., intramuscular) dose of a trivalent (*S*. Enteritidis/*S*. Typhimurium/*S*. Typhi) conjugate vaccine in comparison to a placebo control consisting of buffer plus preservative (2-phenoxyethanol).

In total, approximately 44 healthy eligible participants will be sequentially enrolled into Part 1 of the study in three steps based on dose.

In **Step 1** (Cohort A), 10 participants will be randomly allocated to one of two treatment groups to receive:

- trivalent vaccine, 6.25 mcg of each COPS and Vi, in 0.125 ml volume (N=8) mixed with 0.375 ml of placebo (phosphate buffered saline + 2-phenoxyethanol + Tween 80 stabilizer) to reach a volume of 0.5 ml;
- or placebo, 0.5 ml volume (N=2).

In **Step 2** (Cohort B), 12 participants will be randomly allocated to one of two treatment groups to receive:

- trivalent vaccine, 12.5 mcg of each COPS and Vi, in 0.25 ml volume (N=10) mixed with 0.25 ml of placebo to reach a volume of 0.5 ml;
- or placebo, 0.5 ml volume (N=2).

In **Step 3** (Cohort C), 22 participants will be randomly allocated to one of five treatment groups to receive:

- one dose of trivalent vaccine, 25 mcg of each COPS and Vi, in 0.5 ml volume (N=6);
- one dose of trivalent vaccine, 12.5 mcg of each COPS and Vi, in 0.5 ml volume (N=4);
- two doses of trivalent vaccine, 25 mcg of each COPS and Vi, in 0.5 ml volume (N=6);
- two doses of trivalent vaccine, 12.5 mcg of each COPS and Vi, in 0.5 ml volume (N=4);
- or placebo, 0.5 ml volume (N=2).

Each of the escalated dosage cohorts is designed to be slightly larger in size in order to have a higher probability to detect potential reactogenicity with the increasing quantities of flagellin, which is a component (the protein carrier of the NTS conjugates), while increasing the number of subjects exposed in a step-wise fashion.

Following collection of the 28 day post-vaccination clinical data and rapid measurement of serum IgG ELISA antibody levels, there will be an interim analysis of the safety and immunogenicity in preparation for advancement to Part 2 of the study.

Part 2. The second part of the study is intended to expand the safety and immunogenicity data from a selected dose level of the vaccines. The target dose for this part of the study will be informed by the reactogenicity data from Part 1 of the study with particular attention on the dose level of vaccine administered in Step 3 (administration of the highest dose) and by the immunogenicity data from the dose-escalation cohorts, with an intent to select the most immunogenic yet well-tolerated dose of the vaccine formulations. Based on experiences with existing licensed and unlicensed Vi conjugate vaccines, we anticipate that the trivalent

conjugate vaccine containing 25 mcg of polysaccharide of each conjugate will likely be the most immunogenic dose and will hopefully be as well-tolerated as monovalent Vi conjugate vaccines.

Part 2 of the overall study will intend to enroll 60 eligible participants who will be randomized to one of three possible treatment arms:

- Trivalent conjugate vaccine followed 28 days later by a second dose of trivalent conjugate vaccine (n=25);
- Trivalent conjugate vaccine followed 28 days later by a dose of placebo (n=25);
- A dose of placebo followed 28 days later by a second dose of placebo (n=10).

2.1 How the Trivalent Conjugate Vaccine is expected to be used in sub-Saharan Africa

By administration of a Trivalent Conjugate Vaccine, we will aim to prevent two distinct clinico-epidemiologic forms of invasive salmonellosis that affect somewhat different age groups in sub-Saharan Africa, including invasive disease (septicemia, bacteremia, meningitis, etc.) caused by certain non-typhoidal serovars (in particular, *S*. Typhimurium and its variants, and *S*. Enteritidis) and typhoid fever caused by *S*. Typhi. The *S*. Typhimurium and *S*. Enteritidis conjugates in the Trivalent Conjugate Vaccine are intended to prevent iNTS disease which exhibits a peak incidence from 6-23 months of age.[7,37] The iNTS incidence drops somewhat in children 24-35 months of age and then falls further in those 36-47 months of age.[7,37] Thus, to diminish the iNTS burden the Trivalent Conjugate Vaccine will have to be administered in early infancy to elicit immune responses in time to protect children 6-11 months of age when maternally-transferred antibodies will have waned. The Typbar-TCV component of the Trivalent Conjugate is intended to prevent typhoid fever, a disease that shows a low incidence in infancy but rises in children age 24 months and often peaks in children 5-9 years of age.

The ultimate preferred regimen to administer the Trivalent Conjugate Vaccine will be determined by analysis of the results of future Phase 2 clinical trials that will be carried out in infants and toddlers in sub-Saharan Africa with the doses being administered at ages when infants and toddlers receive other vaccines within the schedule of the Expanded Program on Immunization (EPI). Currently in sub-Saharan Africa, three doses of parenteral pentavalent vaccine (diphtheria and tetanus toxoids, whole cell pertussis vaccine, *Haemophilus influenzae* type b conjugate and hepatitis B combination vaccine) are given to young infants at ~6, ~10 and ~14 weeks of age; another parenteral vaccine administered at these visits is multivalent pneumococcal conjugate vaccine. In sub-Saharan Africa the initial measles containing vaccine is administered at ~9 months of age (along with yellow fever vaccine in certain countries). A second dose of measles containing vaccine is administered at ~15 months of age. Future Phase 2 studies in infants and toddlers will compare the safety, immunogenicity, and practicality of spaced 2-dose and 3-dose regimens of Trivalent Conjugate Vaccine administered during infancy alone or during infancy and toddler age. Moreover, the compatibility of the Trivalent Conjugate with concomitantly administered EPI vaccines will have to be established.

The purpose of the Phase 1 trial in U.S. adults is to gather preliminary safety and immunogenicity data to pave the way for initiating future Phase 2 trials in sub-Saharan Africa. Part 2 of this study in healthy U.S. adults offers a potential opportunity to determine whether at day 56 the anti-COPS and anti-Vi antibodies in participants of the two-dose regimen exhibit

higher avidity and perhaps higher titers than the antibodies at day 56 in recipients of a single dose of Trivalent Conjugate Vaccine.

Serological data demonstrate that a single dose of monovalent Typbar-TCV conjugate administered to Indian children 6-23 months of age is highly immunogenic in stimulating Vi antibodies and the titers remain elevated for up to 5 years post primary-immunization. There are no published or public human data to dictate the serum antibody response to a conjugate vaccine consisting of COPS conjugated to a carrier protein. Moreover, adult humans immunized with a glycoconjugate vaccine of any sort typically mount a maximal antibody response after the first dose that is not boosted following administration of a second dose of glycoconjugate. Therefore, the main purpose of administering two-sequential doses of trivalent conjugate separated by 4 weeks is to evaluate whether a two-dose regimen presents an increased risk for reactogenicity when the second dose is administered. A second dose is not expected to increase anti-COPS titers in U.S. adults but it may perhaps increase antibody avidity.

2.2 Work flow of the Phase 1 study in U.S. adults

Eligible subjects will be screened, enrolled, and vaccinated at the CVD Outpatient Vaccine Clinic (located on the 4th floor of HSF1) or Pharmaron (located in the UMB BioPark 1 building). After the documentation of informed consent, subjects will be screened for eligibility; eligible subjects will subsequently be enrolled and randomized to a treatment group. On Day 1 (and Day 29, for participants in Cohorts C and D), study product will be administered, in double-blinded fashion, as an intramuscular injection into the deltoid muscle. Following vaccination, there will be a 20-minute post-vaccination observation period. Following each dose of study product, safety and reactogenicity will be measured by a daily oral temperature and solicited AE reporting over the subsequent 7 days (via a 7-day memory aid); unsolicited AE reporting will occur through 28 days following the last dose of vaccine. The occurrence of any SAEs will be recorded through 6 months after the last dose of vaccine (Day 180 or 210, for participants in Cohort C and D).

Subjects in Steps 1-2 (Cohorts A-B) will complete four additional outpatient visits on Days 8, 29, 57, and 180 (final visit). Subjects in Cohorts C and D will complete five additional outpatient visits on Days 8, 29 (second dose of masked study product), 36, 57, and 210 (final visit). The recording of AEs and concomitant medication change information, the performance of a physical exam (if indicated), and the collection of study-specific samples will be performed during these outpatient visits. Clinical safety laboratory tests will be evaluated on Day 8. The final study visit will be for the collection of SAE information (Day 180 or 210).

After the completion of enrollment of each cohort, the safety data through 7-days post-vaccination are to be reviewed by an independent Safety Monitoring Committee (SMC). The SMC must provide approval prior to the enrollment of the subsequent dose-escalating cohort. Immunology data may not be available at the times of these scheduled SMC reviews.

In addition to the evaluation of safety, immunogenicity assessments are planned to evaluate the immune responses induced by the three different vaccine formulations. The study, however, is not statistically powered to determine significance of any pre-specified difference. Therefore, there was no formal sample size calculation, based on immunogenicity, as the basis for the designed study. Nonetheless, the sample size was chosen based on the number of subjects appropriate for a phase 1 study in which these vaccines have never been evaluated in humans.

Schematic of Study Cohorts

Cohort	Vaccine	Dose of each COPS and of Vi (µg)	No. Subjects	Dose #1	Dose #2	Vaccination Days
A (Step 1)	Trivalent Placebo	6.25 0	8 2	Vaccine Placebo	- -	1
SMC review of available safety data through 7-days post-vaccination before proceeding to the next dosage cohort						
B (Step 2)	Trivalent Placebo	12.5 0	10 2	Vaccine Placebo	- -	1
SMC review of available safety data through 7-days post-vaccination before proceeding to the next dosage cohort						
C (Step 3)	Trivalent Trivalent Trivalent Trivalent Placebo	25 25 12.5 12.5 0	6 6 4 4 2	Vaccine Vaccine Vaccine Vaccine Placebo	Vaccine Placebo Vaccine Placebo Placebo	1& 29* 1 & (29)* 1& 29* 1 & (29)* 1 & (29)*
SMC review of available safety data through 7-days post-vaccination before proceeding to Part 2 of the study						
D (*Expanded, confirmatory cohort*)	Trivalent Trivalent Placebo	(*highest, well-tolerated dose among cohorts A-C*)	25 25 10	Vaccine Vaccine Placebo	Vaccine Placebo Placebo	1 & 29* 1 & (29)* 1 & (29)*
		Total	N = 104			

* In cohorts C and D, the trivalent vaccine will be administered as a single dose (day 1) or as a two-dose (days 1 and 29) regimen. In order to maintain the masking of study products, on day 29 a placebo will be administered when indicated by the parentheses.

SMC, Safety Monitoring Committee

Schematic of the Flow of the Cohort enrollments and timing of SMC meetings:

Part 1

Step 1 (Cohort A)
single 6.25 µg dose trivalent
or single dose placebo
↴
[**SMC Review**]
↴
Step 2 (Cohort B)
single 12.5 µg dose trivalent
or single dose placebo
↴
[**SMC Review**]
↴
Step 3 (Cohort C)
single 25 µg dose trivalent
or two-dose 25 µg dose trivalent
or single 12.5 µg dose trivalent
or two-dose 12.5 µg dose trivalent
or single dose placebo
↴
[*Go/No-Go Decision for Part 2 based on interim safety and immunogenicity assessments*]
↴
[**SMC Review**]
↴

Part 2

	Cohort D Trivalent on day 1, (placebo on day 29); Trivalent on day 1 and day 29; Or Placebo on day 1 and day 29

2.3 Primary Objectives and Outcome Measures

The primary objectives and outcome measure of this study are the following:

1. Safety and Reactogenicity: To assess the frequency and severity of solicited local (i.e., injective site) and systemic (such as fever) AEs during the first 7 days following each dose of vaccine.
2. Safety: To assess the frequency and severity of unsolicited AEs within 28 days of each dose of vaccine and the occurrence of any SAEs through 6 months after the last dose of vaccine
3. Immunogenicity: To measure the proportion of subjects that achieve a four-fold increase in titer, as compared to baseline, of specific serum IgG anti-COPS (*S*. Enteritidis or *S*. Typhimurium), anti-Vi (*S*. Typhi) polysaccharide, and anti-FliC (*S*. Enteritidis or *S*. Typhimurium) antibody at days 29 and 57, as measured by ELISA.

2.4 Secondary Objectives and Outcome Measures

The secondary objectives and outcome measures of this study are the following:

1. Safety: To assess the frequency and severity of abnormalities in clinical safety laboratory parameters at 7 days following the first dose of vaccine.
2. Immunogenicity: To measure the proportion of subjects that achieve a four-fold increase in titer, as compared to baseline, of specific serum IgG anti-COPS (*S*. Enteritidis or *S*. Typhimurium), anti-Vi (*S*. Typhi) polysaccharide, and anti-FliCn (*S*. Enteritidis or *S*. Typhimurium) antibody at day 180/210, as measured by ELISA.
3. Immunogenicity: To calculate the GMT of serum IgG anti-COPS (*S*. Enteritidis or *S*. Typhimurium), anti-Vi polysaccharide, and anti-FliC (*S*. Enteritidis or *S*. Typhimurium) antibodies at days 1, 29, 57, and 180/210, as measured by ELISA.

2.5 Exploratory Objectives and Outcome Measures

The exploratory objectives and outcome measures of this study include the following:

1. Immunogenicity: To measure antibodies by serum bactericidal antibody (SBA) activity assay and opsonophagocytic activity (OPA) assay against *S*. Typhimurium, *S*. Enteritidis, and *S*. Typhi at days 1, 29, 57, and 180/210.
2. Immunogenicity: To measure the antibody secreting cells (ASC), specific for anti-COPS, Vi, FliC, and TT; possibly including homing markers and the measurement of antibodies in lymphocyte supernatants (ALS)
3. Immunogenicity: To measure memory B and T cells, specific for anti-COPS, Vi, FliC, and TT activity; including the presence of B cell homing markers as assessed by the expression of mucosal homing marker integrin α4β7 and the lymph node homing marker CD62L

4. Immunogenicity: To conduct functional genomic and proteomic studies to further explore the immune response to vaccination.
5. Immunogenicity: To measure the anti-Vi polysaccharide, anti-TT, anti-COPS, and anti-FliC antibody levels in oral fluid at days 1, 29, 57, and 180/210, as measured by ELISA (Cohorts C and D)

3 STUDY POPULATION

The study population will include healthy adult men and women ages 18 to 45 years, inclusive. Participants will be recruited from the local Baltimore-Washington DC population through established methods, to include: future-contact authorized CVD volunteer database, local advertisements, campus flyers, and word-of-mouth. Potential volunteers who are interested in the study are instructed to call the CVD to receive further information about the study.

The process of informed consent begins when a person contacts the CVD for information about the study. CVD research staff receive these inquiries and read through an IRB-approved telephone script which describes basic information on the study topic, duration and number of visits, procedures, and eligibility criteria. No personal health information is solicited and afterwards interested potential participants are given an appointment to attend an *Orientation Session* for formal screening.

At the *Orientation Session*, the consent process continues. CVD research staff provide a detailed description of all aspects of the study, including the rationale and background, the public health significance, the procedures and schedule of visits, and a detailed discussion of the risks and the presence or absence of benefit to them, as appropriate. The prospective subject is given a copy the study consent form (without the signature page) and is encouraged to discuss his/her participation with family members or advisors before agreeing to sign the informed consent form and proceeding with any screening procedures. An interested volunteer may decide to participate in the study on a separate visit or may continue the consent process after the *Orientation Session* is completed.

A similar process of recruitment, pre-screening, and consenting process will be performed at Phamaron. Pharmaron will use their recruitment process which may include participants outside of the local Baltimore-Washington vicinity. Pharmaron will use their established methods of recruitment which will include their volunteer database, local advertisements, social media, and other methods. As part of the consenting process, Pharmaron research staff will provide detailed description of all aspects of the study, including rationale and background, the public health significance, the procedures and schedule of visits, and a detailed discussion of the risks and the presence or absence of benefit to them. The recruitment materials will be UMB IRB approved.

Once both the investigator and the volunteer are satisfied that all questions have been answered and the volunteer expresses the desire to participate, the volunteer will be allowed to sign the consent form. A copy of the signed consent document will be given to the participant.

After the informed consent form is signed, a one-on-one private meeting with a member of the study team is performed, to continue the process of informed consent. A brief written examination is administered to assess the volunteer's comprehension of the study (i.e., *Comprehension Assessment Tool*). A passing score of ≥70% is required; wrong answers will be reviewed with the volunteer and examination may be re-taken once. Upon successful completion of the *Comprehension Assessment Tool*, the research staff can proceed with formal screening.

3.1 Inclusion Criteria

Subjects must meet all inclusion criteria in order to participate in the study:

1. Ability to provide written informed consent
2. Age 18 - 45 years, inclusive
3. Good general health as determined by: vital signs (heart rate <100 bpm; blood pressure systolic >90 mm Hg and ≤150 mm Hg; diastolic >45 mm Hg and ≤90 mm Hg; oral temperature <100.4ºF), medical history, and a physical examination† within 45 days before administration of first dose of vaccine.

   † *The intent is to evaluate for acute or ongoing chronic medical conditions which have been present for 90 days or more and which could affect the assess of safety or immunogenicity. Chronic medical conditions should be stable for at least 60 days; defined as no hospitalizations, ER, or urgent care for medical intervention and no change in chronic prescription medications for at least 60 days. Changes in medications due to insurance or financial reasons and when within the same class of medications or changes for improvements in medical conditions are not exclusionary. Medications which are taken prn are also no exclusionary.*

4. Expressed interest and availability to fulfill the study requirements
5. For females of child-bearing potential*, must agree to acceptable birth control&, 4 weeks before enrollment and through 4 weeks after last vaccination.

   * *females of child-bearing potential are defined as: not sterilized via tubal ligation, bilateral oophorectomy, salpingectomy, hysterectomy, or successful Essure® placement (permanent, non-surgical, non-hormonal sterilization) with documented radiological confirmation test at least 90 days after the procedure, and still menstruating or <1 year of the last menses if menopausal.*

   & *acceptable birth control includes: non-male sexual relationships, abstinence from sexual intercourse with a male partner, monogamous relationship with vasectomized partner who has been vasectomized for 180 days or more prior to the subject receiving the first study vaccination, barrier methods such as condoms or diaphragms with spermicide or foam, effective intrauterine devices, NuvaRing®, and licensed hormonal methods such as implants, injectables, or oral contraceptives.*

6. Agrees not to participate in another clinical trial at any time during the study period.
7. Agrees to allow for the indefinite storage of blood samples for future research use.

3.2 Exclusion Criteria

Subjects who meet any of the following criteria at baseline will be excluded from participating in the study:

1. History of typhoid vaccination or known history of typhoid infection within 5 years
2. Unacceptable laboratory abnormality from screening (prior to first vaccination) or upon safety laboratory testing (prior to second vaccination) as listed below. Laboratories with abnormalities which are possibly transient in nature may be repeated one time.

   a. Hemoglobin, white blood cell (WBC) count, absolute neutrophil count (ANC), or platelet count of an unacceptable value, according to *Appendix B*

b. Creatinine, AST, ALT, total bilirubin, or C-reactive protein of an unacceptable value, according to *Appendix B*

c. Positive serology for hepatitis C or HIV antibody or hepatitis B surface antigen. (*Subjects will be informed if their results are positive for hepatitis C, HIV antibody or hepatitis B surface antigen and will be referred to a primary care provider for follow up of these abnormal laboratory tests*.)

3. For women of child-bearing potential, positive serum pregnancy test (during screening within 45 days of enrollment) or positive urine pregnancy test (prior to and within 24 hours of administering each dose of vaccine).
4. Nursing mother.
5. Temperature > 38.0°C (100.4°F) or symptoms of an acute self-limited illness such as an upper respiratory infection, including of a COVID-19 infection#, or gastroenteritis within 10 days prior to each dose of vaccine.

   # *Symptoms of a COVID-19 infection include fever, chills, cough, shortness of breath or difficulty breathing, fatigue, muscle or body aches, headache, new loss of taste or smell, sore throat, congestion or runny nose, nausea or vomiting, and diarrhea.* https://www.cdc.gov/coronavirus/2019-ncov/symptoms-testing/symptoms.html

6. Medical, occupational, or family problems as a result of alcohol or illicit drug use during the past 12 months.
7. Diagnosis of schizophrenia or other major psychiatric disease
8. Failure to pass *Comprehension Assessment Tool* during screening (70% correct answers are required to pass. Subjects will be provided the opportunity to repeat the test once).
9. Receipt of an experimental agent (vaccine, drug, device, etc.) within 28 days before enrollment or expects to receive an experimental agent during the study period.
10. Receipt of any licensed vaccine within 2 weeks (for inactivated vaccines) or 4 weeks (for live vaccines) before enrollment in this study.
11. Known sensitivity to any ingredient in the study vaccine, including a history of severe allergic reaction to tetanus vaccine.
12. Receipt of immunoglobulin or other blood product within the 3 months prior to vaccination in this study.
13. Immunosuppression as a result of an underlying illness or treatment with immunosuppressive or cytotoxic drugs, or use of anticancer chemotherapy or radiation therapy within the preceding 36 months.
14. Long-term use (>2 weeks) of oral or parenteral steroids (glucocorticoids), or high-dose inhaled steroids (>800 mcg/day of beclomethasone dipropionate or equivalent) within the preceding 6 months (nasal and topical steroids are allowed).
15. Other condition that in the opinion of the investigator would jeopardize the safety or rights of a volunteer participating in the trial or would render the subject unable to comply with the protocol.

3.3 Enrollment and Randomization Procedures

Per International Conference on Harmonisation (ICH) guideline E6: Good Clinical Practice (GCP), screening records will be kept at the clinical site to document the reason why an individual was screened, but failed trial entry criteria. The reasons why individuals failed screening will be recorded in the data system.

3.3.1 Re-screening procedure

There may be situations in which a subject is not able to be randomized within 45 days of his/her Screening Visit. In these instances, the subject may need to be re-screened including re-consent. This will involve undergoing all screening procedures again.

Study participants that meet all inclusion and exclusion criteria and have signed the informed consent form will be assigned a subject identification number. A randomization code will be prepared by a CVD statistician and will determine the blinded assignment of each subject to a treatment arm. A designated independent individual (e.g., Independent Safety Monitor) will be provided with a back-up randomization code list for emergency unblinding purposes and which will be maintained in a secure location.

Subjects, investigators, and study personnel performing any study-related assessments following study vaccine administration will be blinded to subject treatment assignment. Laboratory personnel performing the immunogenicity assessments will also remain blinded to subject treatment assignment.

3.4 Withdrawals and Discontinuation from Study

Participation in the study is strictly voluntary. Participants have the right to withdraw from the study at any time and for any reason, without penalty or prejudice to further treatment. The Principal Investigator and/or designee may, at their own discretion, terminate a subject from continuing in the study if it is considered to be in the participant's best welfare, or if the participant is not willing or able to comply with the study requirements. The reason for withdrawal or termination will be documented. Study participants may voluntarily withdraw their consent for participation at any time and for any reason, without penalty. A study participant may be withdrawn from the study for the following reasons:

- Medical illness or a new clinical finding which in the opinion of the investigator could compromise the safety of the subject or interfere with the evaluation of the safety, tolerability, or immunogenicity of the study products.
- The subject no longer meets eligibility criteria.
- As deemed necessary by the investigator for non-compliance or other reasons.
- Subject lost to follow-up.
- Termination of the study.
- New information becomes available that makes further participation unsafe.

Handling of Participant Withdrawal or Termination. Every effort will be made to undertake protocol-specified safety follow-up procedures to capture AEs, serious adverse events (SAEs), and unanticipated problems (UPs). In the event of withdrawal or termination from the study, reasonable efforts should be made to conduct the following procedures:

- Review diary card/ memory aid, if still in use prior to withdrawal
- Updating any ongoing AE/SAEs that remain ongoing at time of subject's last visit prior to withdrawal
- Query about AEs, SAEs and concomitant medications if the interval between the subject's last visit and the time of withdrawal is within the protocol defined reporting period
- Targeted physical examination, if there are any ongoing AEs at the time of withdrawl
- Blood for safety laboratory testing, if withdrawal occurs before the day 8 visit
- Update contact information

4 INVESTIGATIONAL PRODUCTS

4.1 Description of Investigational Products

4.1.1 Trivalent, with preservative (multi-dose vials)

This study product consists of a cGMP lot of a trivalent *S*. Enteritidis/*S*. Typhimurium/*S*. Typhi Vi conjugate vaccine consisting of a 1:1:1 mix of *S*. Enteritidis COPS linked to Phase 1 Enteritidis flagellin (FliC) subunits and *S*. Typhimurium COPS linked to Phase 1 Typhimurium flagellin (FliC) subunits, plus Typbar TCV® (*S*. Typhi Vi capsular polysaccharide linked to tetanus toxoid protein). The study product is presented in multi-dose vials containing 2-phenoxyethanol preservative.

4.1.2 Placebo

The placebo consists of the buffer vehicle in which the conjugate vaccines are suspended, with 2-phenoxyethanol preservative.

4.2 Formulation, Storage, Packaging, and Labeling

4.2.1 Trivalent, with preservative (multi-dose vials)

Each vial holds 2.5 mL (plus overfill) of material for which a 0.5 mL volume contains *S*. Enteritidis 25 µg (by polysaccharide weight), *S*. Typhimurium 25 µg (by polysaccharide weight), and S. Typhi Vi 25 µg (by polysaccharide weight) conjugate vaccine, plus Tween-80 and PBS, and 2-phenoxyethanol preservative. Each multi-dose vial is to be stored between 2-8°C, not to be frozen.

4.2.2 Placebo

Each vial holds 2.5 mL (plus overfill) of material for which a 0.5 mL volume contains PBS, Tween-80, plus 2-phenoxyethanol preservative. Each multi-dose vial of placebo is to be stored between 2-8°C, not to be frozen.

4.3 Preparation and Administration

Step 1 (Cohort A). For each dose of vaccine, a 0.25 mL volume from a vial containing study product is to be mixed with 0.75 mL of placebo (PBS, Tween-80, and 2-phenoxyethanol). After mixing, 0.5 mL should be withdrawn into a 1 mL syringe -- this volume should contain ~6.25 µg of product (by polysaccharide weight) as a dose in 0.5 mL. The 0.5 mL of placebo will contain only buffer and preservative.

Step 2 (Cohort B) and Cohort C. For each dose of vaccine, a 0.5 mL volume from a vial containing study product is to be mixed with 0.5 mL of placebo (PBS, Tween-80, and 2-phenoxyethanol). After mixing, 0.5 mL should be withdrawn into a 1 mL syringe-- this volume should be equivalent to ~12.5 µg of test product (by polysaccharide weight) dose in 0.5 mL. The 0.5 mL of placebo will contain only buffer and preservative.

Step 3 (Cohort C). For each dose of vaccine, a 0.5 mL volume from a vial containing study product is to be directly withdrawn into a 1 mL syringe-- this should be equivalent to ~25 µg (by polysaccharide weight) dose in a volume of 0.5 mL. The 0.5 mL of placebo will contain only buffer and preservative.

The diluted study product vial can be used up to 4 hours, if stored at 2-8 °C, or up to a maximum of 30 minutes if kept at room temperature. Prepared diluted vaccine must be manually shaken gently before using. Once the mixed vaccine has been drawn into a syringe, it should be administered within 30 minutes. Any unused vaccine or waste material should be disposed of in accordance with local requirements.

Each vial (holding 2.5 mL of material plus overfill) shall only be used to withdraw a maximum of 5 doses (5 punctures per vial) for each day of scheduled administration. Each multidose vial will only be used for one day. At the end of each vaccination day, unused vaccine in the multidose vial will be marked as un-usable. The Pharmacy will be responsible for keeping track of the number of doses drawn per vial and maintaining proper investigational product accountability on the accountability log.

4.4 Study Product Accountability

The study product will be stored in and dispensed by the University of Maryland Medical Center (UMMC) Investigational Drug Service (IDS) pharmacy or Pharmaron. The FDA requires accounting for the disposition of all investigational products. The Investigator is responsible for ensuring that an accurate record of product disposition is maintained and product is dispensed only by authorized personnel as required by applicable regulations and guidelines. Records of product disposition as required by federal law consist of the date received, date administered, quantity administered, and the subject number to whom the study product was administered. The investigational pharmacist will be responsible for maintaining accurate records of the shipment and accountability of the study product.

For participants enrolled at CVD, the preparation and dispensing of the study product is performed by the IDS Pharmacy and the dosing of subjects is performed by CVD research staff; the process and procedures are documented and maintained in a secure location that is segregated from the blinded research staff. Participants enrolled at Pharmaron will have the study product preparation and administration according to their internal standards. The pharmacy records (IDS pharmacy, CVD, and Pharmaron) will be made available for inspection by external monitors and by the relevant regulatory agencies (e.g., FDA) at any time.

4.5 Masking of the Study Product

A prospectively assigned vaccinator and checker will be designated as unblinded personnel and will not have any role in the assessment of adverse events in study participants. Unblinded personnel will be responsible for the preparation of the study products and for the blinded labeling of the study products. The unblinded staff will be responsible for maintaining the randomization key and will secure these study documents in a locked cabinet.

The PI or the Data and Safety Monitoring Board must submit a written request, to inform each other, for emergency unblinding, if this were to be performed. The written request must clearly state the justification for the emergency unblinding and describe which specific participants are to be unblinded. This document will be filed in the study regulatory file.

5 STUDY PROCEDURES

5.1 Clinical Evaluations

Medical History: Will be obtained by interview of the participants. Participants will be queried regarding a history of significant medical disorders of the head, eyes, ears, nose, throat (HEENT), mouth, cardiovascular system, lungs, gastrointestinal tract, liver, pancreas, kidney, urologic, nervous system, blood, lymph glands, endocrine system, musculoskeletal system, skin, and genital/reproductive tract. A history of any allergies, cancer, immunodeficiency, psychiatric illness, substance abuse, and autoimmune disease will be solicited.

Medication History: All current medications and medications taken in the 28 days prior to enrollment (prescription and over-the-counter drugs will be included as well as vitamins and supplements) through 28 days after the last vaccination or early termination (if prior to 28 days from last vaccination), whichever occurs first. Assessment of eligibility also will include a review of permitted and prohibited medications (per the exclusion criteria).

Physical Examination: At screening appropriate study personnel will record oral temperature, pulse and blood pressure and perform a focused physical examination to assess general wellness which will include the following areas/systems: HEENT; lymph nodes; skin; pulmonary; cardiovascular; abdominal; neurological; and musculoskeletal systems. The physical examination should specifically address issues identified by the medical history of the participant. At following visits including enrollment, oral temperature, pulse, and blood pressure will be recorded and a targeted physical examination may be conducted if indicated based on review of medical history.

Reactogenicity Assessments: Will include brief history for assessment of AE/SAEs just prior to and following vaccination (Day 1 for Cohorts A-B and Days 1 and 29 for Cohorts C and D), which includes an assessment of systemic and local reactions. Participants will be observed in-clinic for 20 minutes following each vaccination.

Memory Aids: All participants will complete a subject memory aid distributed after each vaccination. Subject memory aids will be reviewed with the participant for AE/SAEs during visits occurring 7 days after each dose of vaccine. Following review, the appropriate case report form (CRF) will be completed by the study staff. Memory aids will not be retained as a source document.

5.2 Clinical Laboratory Evaluations

A total of 16 mL blood will be collected for screening clinical labs for the evaluation of eligibility. If screening laboratory results are out of range value, they may be repeated once provided there is an alternative explanation for the out of range value. Participants must have acceptable screening laboratory findings prior to enrollment. The acceptable ranges for study eligibility are listed in Appendix B.

For the assessment of safety, a total of 12.5 mL of blood will be collected for clinical safety labs on Day 1 (pre-vaccination) and the Day 8 (7-days post dose #1) visit. This evaluation is to include a CBC with differential for total white blood cells (WBC) count, hemoglobin (Hg), absolute neutrophil count (ANC), platelet count, creatinine, aspartate aminotransferase (AST),

alanine aminotransferase (ALT), total bilirubin (TBIL), and C-reactive protein (CRP). On Day 2 (one day after receipt of the first dose of vaccine), a 3.5 mL volume of blood will be collected for the assessment of C-reactive protein only. The grading of toxicity for the clinical safety lab values that are not in the normal range is provided in Appendix C.

Due to the U.S. Public Health Emergency with COVID-19, SARS-CoV-2 testing will be performed on the days of scheduled vaccination. These test results are not intended to inform the eligibility of participants for vaccination, as the results are likely to be available after 2-3 days. However, for possible pre-symptomatic participants (i.e., eligible due to the absence of symptoms at the time of vaccination) the positive test result could be used to provide an alternate etiology for the development of symptoms due to COVID-19 rather than due to the study product. The test results of acute infection (positive PCR) or prior infection (positive antibody) will also be used in planned stratified analysis of the immunology outcomes, as COVID-19 infection may alter the immune response to vaccination. The results of SARS-CoV-2 testing will be provided to participants at their Day 7 and Day 36 visits. If SARS-CoV-2 testing results are received prior to these visits, the research team may call participants to report these results.

All women of child-bearing potential will have a serum pregnancy test performed during screening and a urine pregnancy test on the vaccination days. The results of the urine pregnancy test must be negative prior to enrollment in the study, and prior to each vaccination.

When a result is abnormal and satisfies the toxicity grading scale, the result must be recorded as a laboratory AE and followed until resolution or it becomes stable. If a laboratory value is abnormal but does not satisfy the toxicity grading scale, then the abnormality must be assessed by the site PI or designee for clinical significance. For all clinically significant abnormalities or grade 1 or higher abnormalities, the value should be recorded as a laboratory AE. Any grade 2 or higher abnormality must be followed until resolution, it becomes stable, or it becomes grade 1 or lower.

5.3 Research Laboratory Assays

Blood volumes have been summarized in *Appendix A* (Schedule of Events). The Immunological Assays to be performed with these blood specimens have been described in Section 8 (Assessment of Immunogenicity).

The collection of oral crevicular fluid will be performed with a porous foam swab device that is gently rubbed along the gumline until the foam sponge is saturated. The foam swab is then inserted into an extraction tube that is prefilled with a buffer solution that elutes the antibodies from the foam swab. A dropper tip, attached to the tube and swab, effectively acts as a syringe-like device which simultaneously squeezes the oral fluid-buffer liquid out of the foam and into the collection tube. Antibodies in oral fluid will be measured by the method described in Section 8.

6 STUDY SCHEDULE

6.1 Screening Visit (Day -45 till -2)

After a participant has signed informed consent and has successfully completed the *Comprehension Assessment Tool*, the following will be performed:

- Assignment of study subject identification number
- Complete medical history will be obtained by interview
- Record all concomitant medications taken within 28 days prior to signing the informed consent form
- Measure oral temperature, blood pressure, and pulse.
- Collect height and weight
- A targeted physical examination is to be performed by a licensed study clinician
- A blood volume of 16 mL is to be obtained for screening laboratories, consisting of:
    - Hematology (4 mL, lavender, EDTA tube): total WBC, ANC, Hg, platelets
    - Chemistries (8.5 mL, red, serum separator tube): creatinine, AST, ALT, TBIL, and CRP
    - Serologies (from same 8.5 mL SST tube for chemistries): hepatitis B surface antigen (HBsAg) and hepatitis C virus (HCV) antibody
    - Human immunodefienciency virus (HIV) antibody (3.5 mL, serum separator tube)
    - For women of child-bearing potential: serum β-HCG (to be analyzed from the specimen obtained for chemistries)

6.2 Visit 1 - Enrollment & Vaccination Dose #1 (Day 1)

A participant that satisfies eligibility will be given an appointment for enrollment. The following activities will be completed prior to vaccination:

- interim medical history or concomitant medications
- A targeted physical examination, if indicated based on review of interim medical history
- Measure oral temperature, blood pressure, and pulse
- Final review of all inclusion and exclusion criteria
- A blood volume of 15 mL for the collection of serum, 80 mL for the collection of peripheral blood mononuclear cells (PBMC), and 12.5 mL for safety laboratory assessment, consisting of:
    - Hematology (4 mL, lavender, EDTA tube): total WBC, ANC, Hg, platelets
    - Chemistries (8.5 mL, red, serum separator tube): creatinine, AST, ALT, TBIL, CRP
    - SARS-CoV-2 antibody test (5 mL), to be performed at University of Maryland Pathology Associates (UMPA)
- Collect nasopharyngeal swab for SARS-CoV-2 PCR test, to be performed at University of Maryland Pathology Associates (UMPA)
- For women of child-bearing potential, a urine pregnancy will be performed
- Collect oral fluid (crevicular fluid)
- Confirmation of ongoing informed consent

Enrollment and randomization may be performed after eligibility has been confirmed. Once randomization has been completed, a prospectively assigned unblinded vaccinator and unblinded checker will check the randomization treatment assignment and prepare the masked study product. The injection site will be cleaned with an alcohol swab. The assigned study product will be administered to the deltoid muscle by intramuscular route. The study product administered, the location, and time of administration will be documented on the appropriate source document.

Following receipt of dose #1 of study product, the participant will be directly observed for at least 20 minutes. The PI (or designee) may determine that a participant requires longer on-site observation or clinical assessment, as needed for significant adverse events. Prior to discharge, the participant will be provided with training on the completion of a *Memory Aid*, given a digital thermometer, and provided follow-up visit information and contact phone numbers for study staff.

6.3 Visit 2 Follow Up (Day 2)

The participant will return to clinic to complete the following:

- Review of the *Memory Aid* and retraining on the use of the *Memory Aid*, if necessary
- Any other unsolicited adverse events (AEs) will be recorded
- A blood volume of 3.5 mL for safety laboratory assessment of CRP
- Prior to discharge, a reminder of the date and time for the next visit will be given to the participant

6.4 Visit 3 Follow Up (Day 8 ±1)

The participant will return to clinic to complete the following:

- Interim medical history or concomitant medications
- A targeted physical examination, if indicated based on review of interim medical history
- Collection and review of the *Memory Aid* for reactogenicity information and completion of the corresponding source document.
- Any other unsolicited adverse events (AEs) will be recorded
- A blood volume 12.5 mL for safety laboratory assessment, consisting of:
    - Hematology (4 mL, lavender, EDTA tube): total WBC, ANC, Hg, platelets
    - Chemistries (8.5 mL, red, serum separator tube): creatinine, AST, ALT, TBIL, CRP
- A blood volume of 40 mL for PBMC (for Cohorts B, C, and D only)
- If available, the result from the PCR testing performed on Day 1 will be provided to the participant
- Prior to discharge, a reminder of the date and time for the next visit will be given to the participant

6.5 Visit 4 Follow Up (for Cohorts A-B) or Vaccination Dose #2 (for Cohorts C and D) (Day 29 ±3)

The participant will return to clinic to complete the following, prior to vaccination:

- Interim medical history or concomitant medications

- A targeted physical examination, if indicated based on review of interim medical history
- Collection of any AEs will be recorded
- A blood volume of 20 mL for serum and 80 mL for PBMC
    - 5 mL for a SARS-CoV-2 antibody test, to be performed at University of Maryland Pathology Associates (UMPA)
- Collect nasopharyngeal swab for SARS-CoV-2 PCR test, to be performed at University of Maryland Pathology Associates (UMPA)
- Collect oral fluid (crevicular fluid)
- For women of child-bearing potential, a urine pregnancy will be performed (for Cohort C and D only)
- Confirmation of eligibility for dose #2, if Cohort C and D

For Cohort C and D, the unblinded vaccinator and unblinded checker will administer the appropriate study product and record the procedure on the respective source document. Following receipt of vaccine, the participant will be directly observed for at least 20 minutes and given instructions on the completion of a *Memory Aid*. Prior to discharge, follow-up visit information and contact numbers will be provided.

6.6 Visit 5 Follow Up, only for Cohort C and D (Day 36 ±1)

The participant will return to clinic to complete the following:

- Interim medical history or concomitant medications
- A targeted physical examination, if indicated based on review of interim medical history
- Collection and review of the *Memory Aid* for reactogenicity information and completion of the corresponding source document.
- Any other unsolicited AEs will be recorded
- A blood volume of 40 mL for PBMC
- The result from the PCR testing performed on Vaccination Dose #2 (Day 29) will be provided to the participant
- Prior to discharge, a reminder of the date and time for the next visit will be given to the participant

6.7 Follow Up, Visit 5 for Cohort A-C, Visit 6 for Cohort C and D (Day 57 ±3)

The participant will return to clinic to complete the following:

- Interim medical history or concomitant medications
- A targeted physical examination, if indicated based on review of interim medical history
- Collection of any AEs (for Cohort C and D only) or SAEs will be recorded
- A blood volume of 15 mL for serum and 80 mL for PBMC
- Collect oral fluid (crevicular fluid)

6.8 Final Follow Up, Visit 6 for Cohort A-B (Day 180 ±14), Visit 7 for Cohort C and D (Day 210 ±14)

The participant will return to clinic to complete the following:

- Interim medical history

- A targeted physical examination, if indicated based on review of interim medical history
- Collection of SAEs will be recorded
- A blood volume of 15 mL for serum and up to 80 mL for PBMC
- Collect oral fluid (crevicular fluid)

6.9 Unscheduled Visits

Unscheduled visits may be performed at the participant's request or by judgement of the PI (or designee) when necessary for the diagnosis and/or management of a finding or AEs. All unscheduled visits are to be documented.

6.10 Early Termination

A participant may withdraw from the study for any reason or the PI (or designee) may withdraw the subject for the welfare of the participant. At this visit, vital signs (oral temp, BP, and pulse) and AE recording will be completed.

7 ASSESSMENT OF SAFETY

7.1 Specification of Safety Parameters

Safety will be assessed by the frequency and severity of:

1. Solicited adverse events, reactogenicity events occurring from the time of each vaccination through 7 days after vaccination, to include:
    a. Local injection site reactions: pain, erythema (redness), induration (swelling), and ecchymosis (bruising).
    b. Systemic reactions: fever, chills, fatigue (tiredness), malaise (general unwell feeling), myalgia (body aches), (arthralgia (joint aches), nausea, and headache.
2. Study product-related unsolicited adverse events, non-serious adverse events occurring from the time of each vaccination through 28 days after vaccination.
3. Study product-related serious adverse events occurring from the time of the first vaccination and through the final visit (or time of early withdrawal, when applicable) for each participant.
4. New-onset chronic medical conditions occurring from the time of the first vaccination and through the final visit (or time of early withdrawal, when applicable) for each participant.

Safety assessment will also include laboratory parameters which will be evaluated at baseline (pre-vaccination) and 7 days after the first dose of vaccine and are also to include: WBC, ANC, Hg, platelet counts, creatinine, AST, ALT, TBIL, and CRP. CRP will also be evaluated at one day after receiving the first dose of vaccine (Day 2).

7.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

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

All AEs including local and systemic reactions not meeting the criteria for “serious adverse events” should be captured on the appropriate CRF. Information to be collected includes event description, time of onset, clinician’s assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis, which would include MD, PA, and Nurse Practitioner, listed on the FDA Form 1572), and time of resolution/stabilization of the event. All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the patient is screened should be considered as baseline and not reported as an AE. However, if it deteriorates at any time during the study, it should be recorded as an AE.

Special Circumstance of COVID-19 Pandemic. While there is a U.S. Public Health Emergency for COVID-19, there is the risk of community transmission of infections. The testing of both anti-SARS-CoV-2 antibody and presence of virus by PCR on the days of vaccination (both dose #1 and #2) are intended to provide the investigators with information on potential asymptomatic or presymptomatic infections at vaccination, but the results will not be available in time prior to vaccination. Therefore, the results are intended to potentially provide alternate etiology for reactogenicity that might be observed post-vaccination which could be due to a COVID-19 infection rather than to the study product. The results of SARS-CoV-2 testing will be provided to participants at their Day 7 and Day 36 visits. If SARS-CoV-2 testing results are received prior to these visits, the research team may call participants to report these results.

Severity of Event: All AEs will be assessed by the clinician using a protocol defined grading system. For events not included in the protocol defined grading system, than the following guidelines will be used to quantify intensity.

- Mild – Grade 1: events require minimal or no treatment and do not interfere with the patient's daily activities.
- Moderate – Grade 2: events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- Severe – Grade 3: events interrupt a patient's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

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

Relationship to Study Product: The clinician's assessment of an AE's relationship to Vaccine is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study product assessed using the terms: related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used.

- Related – The event is temporally related to the administration of the study product and no other etiology explains the event.
- Not Related – The event is temporally independent of study product and/or the event appears to be explained by another etiology.

Serious Adverse Event (SAE): 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,
- a life-threatening adverse event*,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or

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

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

All SAEs will be:

- recorded on the appropriate SAE Form
- followed through resolution by a study physician
- reviewed and evaluated for intensity and causality by a physician listed on the Form FDA 1572 as the Principal Investigator or Sub-Investigator.
- reviewed by the Independent Safety Monitoring Committee and the IRB.

7.3 Halting Rules

If any of the following halting rules are met, the study will not proceed with the remaining enrollment or dose escalation without a review by and recommendation from an Independent Safety Monitoring Committee:

- Two or more subjects in a dosage cohort experiences the same systemic toxicity, except for fever, of grade 3 or above and judged to be associated with vaccine.
- Two or more subjects in a dosage cohort experiences a fever at or greater than grade 3 within 7 days of vaccination.
- Two or more subjects in a dosage cohort experiences the same grade 2 or greater abnormal laboratory value and judged to be associated with vaccine.
- Three or more subjects in a dosage cohort experience the same localized toxicity (local pain or induration) at grade 3 or above judged to be associated with vaccine.
- Any death occurring within 7 days following administration of vaccine that was not the result of trauma or accident.
- Any subject experiences an SAE determined to be related to vaccine.

The study may also be halted due to PI or Sponsor discretion.

7.4 Safety Oversight

Safety oversight will be under the direction an Independent Safety Monitoring Committee (SMC). There will be an Independent Safety Monitor (ISM) who will be independent of the study team, local to the study site and will be provided access to study records, if required. The SMC will be comprised of the ISM plus at least two independent members with expertise in infectious

diseases and/or clinical trials who will advise the Sponsor and the PI. SMC quorum is defined as a simple majority. The SMC will be responsible for three scheduled meetings to review the available data up to 7-days post-first dose of vaccination for each Cohort; for Cohorts A-C, the SMC will determine whether the trial may proceed to the next dosage cohort. The ISM will review all SAEs in real time and provide a written ISM assessment regarding the event. The SMC may receive a final study report, but a recommendation will not be necessary.

8 ASSESSMENT OF IMMUNOGENICITY

8.1 Enzyme-linked Immunosorbent Antibody Assay (ELISA)

The serum IgG levels against the respective antigens will be measured, as previously described.[15,17,18] Briefly, 96-well plates are coated with the respective COPS, Vi, or FliC antigen (5 µg/mL). After overnight incubation, plates are washed and sera are tested in serial dilutions. Specific antibodies will be detected using peroxidase-labeled anti-human IgG and 3,3,5,5-Tetramethylbenzidine (TMB) substrate. Test and control sera are run in duplicate. Titers are calculated by interpolation of absorbance values of test samples into the linear regression curve of a calibrated control (reference serum). The endpoint titers will be reported as ELISA units (EU), which represent the inverse of the serum dilution that produces an absorbance value of 0.2 above the blank. Seroconversion will be defined as a 4-fold increase in the antibody titer after immunization.

8.2 Opsonophagocytic Activity (OPA) assay

The OPA assay is to be performed as previously described[38,39] with some modifications to the originally described Lindow et al. method.[40] Heat-inactivated serum samples will be added to bacteria and incubated for 30 min at 37°C to allow for antibody binding (opsonization). THP-1 macrophage monolayers will be incubated with opsonized bacteria in antibiotic-free medium for 30 min. (OPA) and 24 hr (survival analysis). Following incubation, extracellular bacteria will be killed (using gentamicin) and for the opsonophagocytosis assays, macrophages will lysed and the lysate will be plated onto LB plates, for enumeration of the colony forming units (CFU). For the survival assays, plates will be incubated overnight and the surviving bacteria will be counted by lysing and plating the cell lysates onto LB plates as described above.

8.3 Serum Bactericidal Activity (SBA) assay

The SBA assay is planned to be performed as previously described.[41] Bacteria, from log-phase cultures, are added to a plate for incubation with guinea pig complement and/or baby rabbit complement. Serially diluted heat-inactivated serum samples will be added to the plate. The SBA antibody titer will be defined as the reciprocal of the highest serum dilution that produces>50% killing in relation to the killing observed for the control wells containing bacteria and complement only (i.e., no serum). The titers will be determined from the mean bacterial count from triplicate wells.

8.4 Antibody Secreting Cells (ASC) assay

Plasmablasts secreting antibodies that recognize the respective COPS, Vi, FliC, or TT antigen will be measured using ELISPOT, using previously described methods.[42] The frequency of spot-forming cells (SFC) from replicate wells will be determined and reported as ASC/10^6 PBMC. A positive response will be defined as ≥8 ASC/10^6 PBMC. Antibodies in lymphocyte supernatants (ALS) from these plasmablasts may also be collected and tested using the ELISA assay. From some PBMCs there may be the measurement of the mucosal homing potential of plasmablasts. PBMC will be enumerated by flow cytometry based on molecules expressed in naïve (B_n;

CD19+ IgD+ CD27-) and memory B (B_M; CD19+ IgD- CD27+) cells which direct them to home to distinct sites (e.g., integrin α4β7, CD62L, CXCR3).

8.5 Circulating B cell memory cells (BM) and Cell-mediated Immunity (CMI) (cytokines, T cell effector and memory (including circulating T follicular/helper cells -cT_{fh}-), T cell homing

Blood will be drawn before each vaccination (Days 1, 29, and 57) and on Days 85 and 209 post-vaccination and isolated PBMC cryopreserved for studies of specific BM[43-45] and CMI[46-50] responses, including cytokine production (e.g., interferon-γ) and expression of homing molecules in effector and memory T cells which might direct them to either mucosal or systemic effector sites. Antigen specific cytokine-production and induction of effector and memory T cells, as well as cT_{fh}, will be measured in cryopreserved PBMC stimulated with the appropriate antigens (e.g., FliC, tetanus toxoid). Antigen-stimulated PBMC will be stained for conventional flow cytometry (using fluorochrome-conjugated monoclonal antibodies) or mass cytometry (using metal-conjugated monoclonal antibodies), run in the corresponding instruments, and the data analyzed using supervised and non-supervised flow cytometry analytical packages. The frequency of BM specific for COPS, Vi, FliC, and tetanus toxoid antigens will be measured by a previously described ELISPOT-based assay.

8.6 Antibodies in Oral Fluid

As opposed to IgA-rich saliva, oral crevicular fluid (the fluid found in the crevicle between the tooth and the gum) is a transudate of serum that is ~1% of the IgG concentration of the blood. Using the same method of measure serum ELISA antibody, the antibodies in oral fluid will be measured against Vi polysaccharide, TT, FliC, and COPS. (reference: Tapia MD, asetti MF, Cuberos, L, et al. *Pediatr Infect Dis J* Sept 2006; 25(9): 819-825)

9 QUALITY AND MONITORING

9.1 Quality Control and Quality Assurance

All clinical trials conducted by the University of Maryland Center for Vaccine Development (CVD) are internally audited for quality assurance (QA) quality control (QC) under the CVD-Wide Quality Management Plan (QMP). The purpose of the QMP is to describe QA/QC procedures designed to ensure site and investigator compliance with applicable regulations, adherence to the IRB-approved protocol, generation of credible, quality data, protect data integrity, and safeguard the safety and well-being of study participants. The plan has the authority to enforce and correct clinical and laboratory deficiencies observed during the conduct of a trial/research study.

Quality Management (QM) activities will proceed in compliance with E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1), March, 2018. Consistent with the University of Maryland Center for Vaccine Development site-wide QMP, a Protocol-specific Quality Management Plan (QMP) (Appendix B of the site-wide QMP) of the will be developed for this protocol. The Protocol-specific plan applies a risk-based approach to Quality Management (QM) focused on the essential elements of clinical trial conduct, i.e., recruitment strategies, the informed consent process, eligibility, study-related procedures, clinical and safety assessments, AEs/SAEs, study product management, specimen management, and data management. The plan will also outline site delegation of QC/QA responsibilities, frequency of regulatory file reviews, the audit schedule (e.g., sample size, audit frequency, critical data that will be reviewed, communication, etc.), documentation and reporting of QA findings/queries, and the development of corrective action preventive action (CAPA) plans (as indicated).

Pharmaron will implement a site-specific QMP, similar to the CVD's QMP and following the CVD's standards.

9.2 Clinical Monitoring

Site monitoring is conducted to ensure that the rights and well-being of human subjects are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently IRB-approved protocol/amendment(s), with GCP, and with applicable regulatory requirement(s). Monitoring refers to the methods used by sponsors of investigational studies, or Contract Research Organizations (CROs) delegated site monitoring responsibilities, to oversee the conduct of, and reporting of data from, clinical investigations. Site monitoring includes ensuring appropriate clinical investigator supervision of study site staff and third party contractors.

In order to ensure protocol compliance, monitoring visits by a sponsor-designated professional or monitor will occur at scheduled intervals. The visit frequency will be defined in a monitoring plan (refer to Section 8.1 below) and communicated before study start to the principal investigator and all other appropriate study personnel.

9.3 Site Monitoring Plan

Independent site monitoring will be conducted to assess the progress of the clinical trial and ensure that the rights, safety, and well-being of study participants are protected, study procedures, laboratory procedures, study intervention administration, and data collection processes are of high quality that comply with ICH/GCP, federal, state, and local regulatory authorities and that the study is conducted in accordance with the IRB-protocol and sponsor standard operating procedures (SOPs). Clinical monitoring of this protocol will be performed by qualified individuals selected by the sponsor in accordance with ICH/GCP (Section 5.18.2) and as detailed in the monitoring plan.

Site visits will be made at standard intervals as defined by the sponsor. Monitoring visits will performed in accordance with ICH/GCP (Section 5.18) and will include, but are not limited to, review of regulatory files, participant study charts inclusive of DCFs and source documents contained therein, informed consent forms, study product accountability records, medical and laboratory reports, etc. Study monitors will meet with investigators to discuss visit findings, any problems and/or issues and actions to be taken, if any. The principal investigator will be responsible for review, follow-up and resolution of monitoring findings

10 STATISTICAL CONSIDERATIONS

10.1 Study Hypothesis and Sample Size Considerations

The overarching hypothesis of this phase 1 study is that the trivalent *S*. Enteritidis/*S*. Typhimurium/*S*. Typhi Vi conjugate vaccine will be well-tolerated at all three dosage levels tested and that the highest dosage level will elicit the strongest serum anti-COPS, anti-FliC and anti-Vi antibody responses in humans. The primary hypothesis of safety will be fulfilled if no participants develop a vaccine related SAE and each vaccine is well-tolerated. The secondary hypothesis of immunogenicity will be fulfilled if each vaccine elicits an immune response by ELISA.

The trial sample size is intended to minimize the number of subjects exposed to each vaccine until sufficient safety data have accumulated to proceed to larger trials. The phase 1 trial sample size was not derived to achieve a pre-specified statistical power. Instead, the sample size was selected to descriptively evaluate the safety and immunogenicity of these vaccines in a first-in-man trial. As a result, there will be sufficient power to detect only very large differences in immunological responses among groups, and confidence limits will be fairly wide.

The endpoint measures for the primary, secondary and exploratory objectives are listed in Sections 6.1, 6.2 and 6.3.

10.1.1 Sample size in Part 2 of the study

The adaptive design for the Part 2 portion of this study will involve the evaluation of single dose vs. two-dose regimens of the trivalent vaccine, in comparison to placebo. There is a theoretical possibility of immunological "interference" with the addition of antigens in multivalent glycoconjugate vaccines.[51] Under such circumstances, the responses to some antigens may be reduced. There is no expectation for there to be differences in the seroconversion rates between single dose and two-dose regimens of glycoconjugate vaccines in healthy U.S. adults.[52] On the other hand, the design of Part 2 of the study will substantiate moving forward with two-dose regimens in sub-Saharan Africa where we do expect that more than a single dose will be required in the target pediatric population[53,54]—so the purpose is the demonstration of safety with two sequential doses and not for showing differences in immune responses.

10.2 General Statistical Principles

Descriptive Statistics. To summarize safety, the number and percentage of participants in each cohort that experiences each AE, categorized by body system and preferred term, will be tabulated with by vaccine received along with a corresponding two-sided exact 95% confidence interval (95%CI). For seroresponse rate endpoints, two-sided 95%CI for each proportion will be calculated. When determining GMT antibody titer, the log values will be used to construct the 95%CI using the t-distribution for the mean difference between three pair-wise vaccine groups. The mean difference and the corresponding CI limits will then be exponentiated to obtain the GMT ratio and corresponding CI. If the values deviate from a log normal distribution, then a non-parametric method may be used for the analysis of GMT.

Continuous Measures of Immune Response. Continuous variables of interest that are not normally distributed will be transformed if needed. To compare each vaccine dose group with the placebo group, t test or Mann-Whitney U test will be used for continuous variables as appropriate

Multiple Comparisons. No formal statistical adjustment for multiple comparisons is planned. The investigators recognize that the study involves multiple comparisons (among vaccine doses, between vaccines, etc.) of safety and immunogenicity parameters, and that statistically significant results could be obtained in some cases when there is no difference between the groups being compared. However, such instances (type I errors) should be infrequent; e.g., if we use $p < 0.05$ as the criterion for statistical significance, only 5% of all comparisons that are actually equivalent will be significant. Moreover, it is difficult to know exactly which comparisons should be included in an adjustment. (We would not want to include safety assessments, because the error of assessing a safe vaccine as unsafe is preferable to thinking that a truly unsafe vaccine is safe.) The main purpose of this trial is to provide data for making decisions about further study of this vaccine, and statistical significance per se is not the only criterion for decision-making in this process. Nevertheless, the investigators will keep the issue of multiple comparisons in mind when interpreting the data and making decisions, even though no formal adjustment is made.

Analyzable Subjects. All vaccinated subjects will be included in the reactogenicity analysis even if they have missing data points. All vaccinated subjects will be included in the immunogenicity analysis if they donated at least one pre-vaccination and one post-vaccination specimen.

COVID-19. For participants in Cohort C, COVID-19 testing (both PCR and antibody) will be performed. Because of the potential for COVID-19 infection (either acute infection or recent prior infection) to alter immune responses, there will be the intent to stratify the analysis according to positive or negative COVID-19 infection.

10.3 Go/No-Go Milestones

A set of criteria are to be defined for the safety and tolerability of each of the dose-escalation cohorts (Cohorts A-C or Steps 1-3), in order to proceed to the next higher dosage cohort.

Any dose of vaccine which results in more than one episode of a grade 2 or higher solicited local or systemic adverse event of the same category, may be considered too reactogenic for dose-escalation, unless the SMC recommends otherwise.

A set of *a priori* safety and immunologic benchmarks for the dose-selection of the vaccines in Part 2 of the study (Cohort D) is defined. The highest well tolerated dosage (no more than one grade 2 or higher solicited symptom of the same category) will be selected for further evaluation in Part 2 of the study. Furthermore, the serum antigen-specific ELISA IgG response rates will also be evaluated for dosage selection. Optimally, the final targeted dosage will result in at least 70% response rates with the target dosage of vaccine.

11 ETHICS/PROTECTION OF HUMAN SUBJECTS

11.1 Ethical Standard

The principal investigator will ensure that this trial is conducted in full conformity with the principles of the Bellmont Report: Ethical Principles and Guidelines for the Protection of Human Subjects of Research of the National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research (April 18, 1979) and codified in 45 CFR 46, 21 CFR 50 and 56, and ICH E6; 62 Federal Regulations 25691 (1997), if applicable. The principle investigator's institution (University of Maryland, Baltimore, UMB) will hold a current Federal Wide Assurance (FWA) issued by the Office of Human Research Protection (HRPO).

11.2 Institutional Review Board

The local institution must provide for the review and approval of this protocol and the associated informed consent documents by an appropriate ethics review committee or Institutional Review Board (IRB). Any amendments to the protocol or consent materials must also be approved before they are placed into use unless it is in the best interest of the subjects' safety to implement changes prior to approval. In both the United States and in other countries, only institutions holding a current U. S. Federal-Wide Assurance issued by the Office for Human Research Protections (OHRP) may participate.

Refer to: http://www.hhs.gov/ohrp/assurances/

Prior to enrollment of subjects into this clinical study, the protocol and the informed consent form(s) will be reviewed and approved by the appropriate IRB. Any amendments to the protocol or consent materials will also be reviewed and approved by the appropriate IRB. The responsible official for the IRB will sign the IRB letter of approval of the protocol prior to the start of this clinical study. Should amendments to the protocol be required, the amendments will be submitted to the IRB; an IRB letter of approval of the amendment must be obtained prior to acting upon the amendment in the protocol.

The contact information for the local IRB and the Human Research Protections Office (HRPO) is:

University of Maryland, Baltimore
Human Research Protections Office
620 W. Lexington Street, Second Floor
Baltimore, MD 21201, U.S.A.
Phone 1-410-706-5037

11.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the participant. Consent forms describing in detail the study, study procedures and risks are given to the potential participant and written documentation of informed consent is required prior to starting any study procedure. Consent forms will be IRB-approved and the participant will be asked to read and review the document. Upon reviewing the document, the investigator will explain the research study to the

participant and answer any questions that may arise. The participants will sign the informed consent document prior to any procedures being done specifically for the study. The participant should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participant for their records. The rights and welfare of the participant will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study

11.4 Exclusion of Women, Minorities, and Children (Special Populations)

This clinical study will include women, children of 18 years of age and older, and all minorities who meet the inclusion/exclusion criteria, regardless of religion, sex, or ethnic background.

11.5 Subject Compensation

Thoughtful consideration has been made toward subject compensation for healthy volunteer participants enrolled into clinical studies involving non-licensed vaccines and experimental infections. The overarching ethical issues which the CVD's compensation scheme attempts to address are: (1) avoidance of "undue inducement", (2) payments which may result in economically disadvantaged populations bearing an overwhelming share of the risks and burdens of research, and (3) payments which may violate the ethical norms of the investigator-subject relationship by turning it into a commercial relationship. The compensation scheme for this study is adopted from the Wage-Payment Model.[55] Under the Wage-Payment Model, a participant is assumed to require little skill, but does require time, effort, and an expectation of experiencing undesirable or uncomfortable procedures. Therefore, the CVD's compensation rates are calculated to approximate the hourly wages of an unskilled high-risk laborer. The compensation rate used in this study is an standard rate that is used across all of CVD's clinical research. This compensation rate is clearly described in the informed consent form and is unlikely to be unduly coercive.

Subjects at the Pharmaron site will be compensated for their time and participation.

Subject Confidentiality

Subject (participant) confidentiality is strictly held in trust by the investigators, their staff, and the Sponsor. This confidentiality is extended to cover testing of biological samples and other testing in addition to the clinical information relating to participant.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

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

Participant identity data will be contained in paper study records which will be kept in a locked file cabinet and in a secure electronic database, accessible only to authorized users at each clinical site. The study database will be user-restricted and password-protected. The study database will identify participants by a coded study Volunteer ID number assigned by clinical site personnel, thus participants will not be identified by name.

Subject confidentiality and the limits for access to records is described in the informed consent form.

11.6 Future Use of Stored Specimens

It is intended that any remaining specimens at the closure of the study will be stored at the CVD indefinitely and may be used for further immunological analyses. Participants may choose to have their specimens destroyed and must do this in a written request. This is described in the consent form.

12 DATA HANDLING AND RECORD KEEPING

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

Source data will be generated by the site and collected on data collection forms. Source data will be entered into a REDCap data management system, managed by the Clinical and Translational Research Informatics Center (CTRIC), University of Maryland School of Medicine. REDCap is a secure web-based application for building an electronic database that provides a 21 CFR Part 11, FISMA, and HIPAA-compliant environment. Quality control audits of all key safety, laboratory, and clinical data in the database will be made after data entry has been completed. Coexistent medical conditions, adverse events and other medical events will be coded using MedDRA dictionary. Concomitant medications will be coded using WHO-DD dictionary. When the database has been declared to be complete and accurate, the database will be locked. Any changes to the database after that time will only be by joint written agreement of the study team.

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

The sponsor and/or its designee will provide guidance to the site principal investigators and other study personnel on making corrections to the data collection forms.

13 PUBLICATION POLICY

All investigators funded by the Wellcome Trust must agree to their Open Access Policy. https://wellcome.ac.uk/funding/guidance/open-access-policy

The Open Access Policy contains the following elements:

1. Expect authors of research papers, monographs and book chapters to maximize the opportunities to make their results available for free
2. Require electronic copies of any research papers that have been accepted for publication in a peer-reviewed journal, and are supported in whole or in part by Wellcome Trust funding, to be made available through PubMed Central (PMC) and Europe PMC as soon as possible and in any event within six months of the journal publisher's official date of final publication (similarly, monographs and book chapters must be made available through PMC Bookshelf and Europe PMC with a maximum embargo of six months)
3. Expect Wellcome-funded researchers to select publishing routes that ensure the work is available immediately on publication in its final published form, wherever such options exist for their publisher of choice and are compliant with our policy
4. Will provide grantholders with additional funding to cover open access charges, where appropriate, in order to meet our requirements
5. Encourage – and where it pays an open access fee, require – authors and publishers to license research papers using the Creative Commons Attribution license (CC-BY) so they may be freely copied and re-used (for example, for text- and data-mining purposes or creating a translation), provided that such uses are fully attributed (CC-BY is also the preferred license for monographs and book chapters)
6. Affirm the principle that it is the intrinsic merit of the work, and not the title of the journal or the publisher with which an author's work is published, that should be considered in making funding decisions.

Therefore, following completion of this clinical trial, the investigators intend to publish the results of this research in a peer-reviewed scientific journal, within 12 months of locking the database. The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry, such as ClinicalTrials.gov* (http://clinicaltrials.gov/), which is sponsored by the National Library of Medicine.

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APPENDIX A: SCHEDULE OF EVENTS (COHORT A-B)

Type of Visit	Screen	V	F	F	F	F	F
Study Day	**-45 to -2**	**1**	**2**	**8**	**29**	**57**	**180**
Window				**±1**	**±3**	**±3**	**±14**
Informed Consent	X	X					
Review Eligibility	X	X					
Medical History	X	X					
Targeted Exam, if indicated	X	X					
Vital Signs (temp, HR and BP)	X	X					
Pregnancy Test	X^S	X^U					
Concomitant Meds	X	X					
Randomization		X					
Vaccination		#1					
7-day Memory Aid (disperse & collect)		d	x	c			
AE assessment		X	X	X	X		
SAE assessment		X	X	X	X	X	X
Screening Labs[1]	16 mL						
Safety Labs[2]		12.5 mL	3.5 mL	12.5 mL			
Research Blood Draw – serum		15 mL			15 mL	15 mL	15 mL
Research Blood Draw – PBMC		80 mL		40 mL*	80 mL	80 mL	80 mL
Total cumulative volume of blood, mL	16	123.5	127	139.5 179.5*	234.5 274.5*	329.5 469.5*	424.5 464.5*

Outpatient clinic visit: V= Vaccination; F= Follow-up visit;

Pregnancy testing, by S= Serum pregnancy test; U= Urine pregnancy test

1 - Screening Laboratory testing will include: 4 mL for Hematology (WBC, ANC, Hemoglobin, Platelet count); 10 mL for Chemistry (creatinine, AST, ALT, total bilirubin, and C-reactive protein; inclusive of β-HCG, when indicated); 5 mL for Serology (Hepatitis B surface antigen and Hepatitis C antibody); and 3.5 mL for HIV antibody.

2 - Clinical Safety Laboratory testing on Day 1 and 8 will include: 4 mL for Hematology (WBC, ANC, Hemoglobin, Platelet count) and 10 mL for Chemistry (creatinine, AST, ALT, total bilirubin, and C-reactive protein). On Day 2, 3.5 mL for C-reactive protein only.

* for Cohort B, on the day 8 visit, a 40 mL volume of blood will be drawn for PBMC to allow for ASC and homing studies

APPENDIX A: SCHEDULE OF EVENTS (COHORT C AND D)

Type of Visit	Screen	V	F	F	V	F	F	F
Study Day	-45 to -2	1	2	8	29	36	57	210
Window				±1	±3	±1	±3	±14
Informed Consent	X	X			X			
Review Eligibility	X	X			X			
Medical History	X	X			X			
Targeted Exam, if indicated	X	X			X			
Vital Signs (temp, HR and BP)	X	X			X			
Pregnancy Test	X[S]	X[U]			X[U]			
Concomitant Meds	X	X			X			
Randomization		X						
Vaccination		#1			#2			
7-day Memory Aid (disperse & collect)		d	x	c	d	c		
AE assessment		X	X	X	X	X	X	
SAE assessment		X	X	X	X	X	X	X
Collect oral fluid		X			X		X	X
Collect NP swab, for SARS-CoV-2 PCR test		X			X			
Screening Labs[1]	16 mL							
Safety Labs[2]		12.5 mL	3.5 mL	12.5 mL				
SARS-CoV-2 antibody test		5 mL			5 mL			
Research Blood Draw – serum		15 mL			15 mL		15 mL	15 mL
Research Blood Draw – PBMC		80 mL		40 mL	80 mL	40 mL	80 mL	80 mL
Total cumulative volume of blood, mL	16	128.5	132	184.5	284.5	324.5	419.5	514.5

Outpatient clinic visit: V= Vaccination; F= Follow-up visit;

Pregnancy testing, by S= Serum pregnancy test; U= Urine pregnancy test

1 - Screening Laboratory testing will include: 4 mL for Hematology (WBC, ANC, Hemoglobin, Platelet count); 10 mL for Chemistry (creatinine, AST, ALT, total bilirubin, and C-reactive protein; inclusive of β-HCG, when indicated); 5 mL for Serology (Hepatitis B surface antigen and Hepatitis C antibody); and 3.5 mL for HIV antibody.

2 - Clinical Safety Laboratory testing on Day 1 and 8 will include: 4 mL for Hematology (WBC, ANC, Hemoglobin, Platelet count) and 8.5 mL for Chemistry (creatinine, AST, ALT, total bilirubin, and C-reactive protein). On Day 2, 3.5 mL for C-reactive protein only.

NP= nasopharyngeal swab

APPENDIX B: CLINICAL LABORATORY VALUES, FOR STUDY ELIGIBILITY

Laboratory Parameter	Units of measure	Reference Range*	Study Inclusion
Total white blood cell count, WBC	cells $x10^3$ / mm^3	4.0 – 10.0	3.0 – 11.5
Absolute Neutrophil Count, ANC	cells / mm^3	1560 – 8100	1100 – 8910
Hemoglobin, Hg (male)	gram / dL	13.5 – 17.5	11.0 – 17.6
Hemoglobin, Hg (female)	gram / dL	12.0 – 16.0	10.3 – 17.6
Platelet count	platelet x 10^3 / mm^3	130 - 400	125 - 480
Creatinine (male)	mg / dL	0.7 – 1.3	<1.4
Creatinine (female)	mg / dL	0.6 – 1.1	<1.2
Alanine aminotransferase, ALT	U / L	7-45	<68
Aspartate aminotransferase, AST (male)	U / L	15 – 40	<60
Aspartate aminotransferase, AST (female)	U / L	13 - 30	<45
Total bilirubin	mg/dL	0.2 – 1.2	<1.5
C-reactive protein	mg/dL	<0.8	<1.1
Hepatitis B surface antigen, HBsAg	n/a	Negative	Negative
Hepatitis C Virus antibody	n/a	Non-reactive	Non-reactive
Human Immunodeficiency Virus antibody	n/a	Non-reactive	Non-reactive

* The reference ranges provided are from Garcia Labs, published from 28 July 2016.

APPENDIX C: GRADING SCALES FOR LABORATORY TOXICITY AND REACTOGENICITY

Parameter	Mild, Grade 1	Moderate, Grade 2	Severe, Grade 3
WBC, high (cells x10^3/mm^3)	11.5 – 13.0	13.1 – 15.0	> 15.0
WBC, low (cells x10^3/mm^3)	2.5 – 3.0	1.5 – 2.4	< 1.5
ANC, high (cells/mm^3)	8900 – 10,000	10,010 – 12,000	> 12,000
ANC, low (cells/mm^3)	800 - 1000	600 - 799	< 600
Hemoglobin, high (gram/dL)	< 1.2 x ULN	1.2-1.5 x ULN	> 1.5 x ULN
Hemoglobin, low (gram/dL)	> 0.8 x LLN	0.8 – 0.7 x LLN	< 0.7 x LLN
Platelets, high (x 10^3/mm^3)	< 1.3 x ULN	1.3-1.6 x ULN	> 1.6 x ULN
Platelets, low (x 10^3/mm^3)	> 0.9 x LLN	0.7-0.9 x LLN	< 0.7 x LLN
Creatinine, high (mg/dL)	< 1.3 x ULN	1.3-1.8 x ULN	>1.8 x ULN
ALT, high (U/L)	< 2.5 x ULN	2.5-5.0 x ULN	> 5.0 x ULN
AST, high (U/L)	< 2.5 x ULN	2.5-5.0 x ULN	> 5.0 x ULN
Total bilirubin	< 2.0 x ULN	2.0-5.0 x ULN	> 5.0 x ULN
C-reactive protein	< 2.0 x ULN	2.0-5.0 x ULN	> 5.0 x ULN
Pain, Injection site	Minimal limitation of limb use	Greater than minimal limitation of limb use	Inability to use limb or perform daily activities
Erythema, Injection site*	< 5 cm	5 - 10 cm	> 10 cm
Ecchymosis, Injection site	< 5 cm	5 - 10 cm	> 10 cm
Induration, Injection site	< 5 cm	5 - 10 cm	> 10 cm
Chills	No interference with daily activity	Some interference with daily activity	Significant interference, prevents daily activity
Fatigue			
Headache			
Malaise			
Myalgia			
Arthralgia			
Nausea			
Oral temperature (fever)	100.0 – 101.1°F	101.2 – 102.0 °F	> 102 °F

ULN – upper limit of normal; LLN – lower limit of normal

* Injection site erythema will not count toward a halting rule. Injection site erythema of any size with ulceration, drainage, sterile abscess, or evidence of secondary infection should be recorded as an AE