

CLINICAL STUDY PROTOCOL H03_04TP

Version 1.0

A Phase 2a, Observer Blind, Randomized, Controlled, Single Center Study To Evaluate
The Safety, Reactogenicity And Immunogenicity Of 2 Doses Of The SBVGH
1790GAHB Vaccine Against *Shigella Sonnei*, Administered Intramuscularly In Adult
Subjects From A Country Endemic For Shigellosis

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TABLE OF CONTENTS

TABLE OF CONTENTS.....	2
PROTOCOL SYNOPSIS H03_04TP	8
LIST OF ABBREVIATIONS	23
1. BACKGROUND AND RATIONALE	25
1.1 Background.....	26
1.2 Rationale	27
2. OBJECTIVES.....	28
2.1 Primary Objective(s).....	28
2.2 Secondary Objective(s).....	28
2.3 Exploratory Objective(s).....	28
3. STUDY DESIGN	29
3.1 Overview of Study Design.....	29
3.2 Study Period.....	30
3.3 Blinding Procedures.....	30
3.4 Data Collection	31
3.4.1 Data Collected from Subjects	31
3.4.2 Tools Used for Data Collection	31
3.5 Collection of Clinical Specimens	32
3.6 Stopping/Pausing Guidelines.....	33
3.7 Procedures related to neutropenia.....	34
3.8 Data Monitoring Committee.....	35
3.9 Premature Withdrawal from Study	36
3.10 End of Study	39
4. SELECTION OF STUDY POPULATION.....	40
4.1 Inclusion Criteria	40
4.2 Exclusion Criteria	40
4.3 Criteria for Delay of Vaccination	42
5. STUDY PROCEDURES.....	43

5.1	Pre-vaccination Clinic Visit(s)	43
5.1.1	Informed Consent/Assent	43
5.1.2	Screening	44
5.1.3	Enrolment	45
5.1.4	Randomization	46
5.2	Vaccination Clinic Visit(s)	46
5.2.1	Post-vaccination Procedures	47
5.2.2	Post-vaccination Reminders	47
5.3	Post-vaccination Visit(s)	47
5.3.1	Follow-up Home Visit(s)	47
5.3.2	Follow-up Clinic Visit(s)	48
5.3.3	Safety Follow-up Calls	49
5.4	Unscheduled Visits	49
5.5	Study Termination Visit	49
5.5.1	Early Termination Visit	50
6.	TREATMENT OF SUBJECTS	52
6.1	Study Vaccine(s)	52
6.2	Non-Study Vaccines	53
6.3	Vaccine Preparation and Administration	53
6.4	Vaccine Administration Error or Overdose of Vaccine	54
6.5	Prior and Concomitant Medications and Vaccines	54
6.6	Vaccine Supply, Labeling, Storage and Tracking	55
7.	ASSESSMENTS	57
7.1	Safety Assessment	57
7.1.1	Solicited Adverse Events	57
7.1.2	Unsolicited Adverse Events	60
7.1.3	Evaluation of Adverse Events	60
7.1.4	Serious Adverse Events	62
7.1.4.1	Adverse Events of Special Interest	63

7.1.5	Methods for Recording Adverse Events and Serious Adverse Events.....	64
7.1.5.1	Post-Study Events.....	64
7.1.6	Pregnancies.....	64
7.1.7	Safety Laboratory Measurements.....	65
7.2	Efficacy Assessment.....	65
7.3	Immunogenicity Assessment.....	66
8.	STATISTICAL CONSIDERATIONS.....	67
8.1	Endpoints.....	67
8.1.1	Primary Endpoint(s).....	67
8.1.1.1	Primary Safety Endpoint(s).....	67
8.1.1.2	Primary Efficacy Endpoint(s).....	67
8.1.1.3	Primary Immunogenicity Endpoint(s).....	67
8.1.2	Secondary Endpoint(s).....	67
8.1.2.1	Secondary Safety Endpoint(s).....	67
8.1.2.2	Secondary Efficacy Endpoint(s).....	67
8.1.2.3	Secondary Immunogenicity Endpoint(s).....	68
8.1.3	Exploratory Endpoint(s).....	69
8.1.3.1	Exploratory Safety Endpoint(s).....	69
8.1.3.2	Exploratory Efficacy Endpoint(s).....	69
8.1.3.3	Exploratory Immunogenicity Endpoint(s).....	69
8.2	Success Criteria.....	69
8.2.1	Success Criteria for Primary Objective(s).....	69
8.2.1.1	Success Criteria for Primary Safety Objective(s).....	69
8.2.1.2	Success Criteria for Primary Efficacy Objective(s).....	69
8.2.1.3	Success Criteria for Primary Immunogenicity Objective(s).....	69
8.2.2	Success Criteria for Secondary Objective(s).....	69
8.2.2.1	Success Criteria for Secondary Safety Objective(s).....	70
8.2.2.2	Success Criteria for Secondary Efficacy Objective(s).....	70
8.2.2.3	Success Criteria for Secondary Immunogenicity Objective(s).....	70

8.3	Analysis Sets.....	70
8.3.1	All Enrolled Set	70
8.3.2	All Exposed Set	70
8.3.3	Safety Set.....	70
8.3.4	Full Analysis Set (FAS) Immunogenicity Set	71
8.3.5	Per Protocol (PP) Set Immunogenicity Set.....	71
8.3.6	Other Analysis Sets.....	71
8.3.7	Subgroups	72
8.3.8	Protocol Deviations	72
8.4	Statistical Analysis Plan	72
8.4.1	Analysis of Demographic and Baseline Characteristics	72
8.4.2	Analysis of Primary Objective(s)	72
8.4.2.1	Analysis of Primary Safety Objective(s).....	72
8.4.2.1.1	Analysis of Extent of Exposure.....	73
8.4.2.1.2	Analysis of Solicited Local, Systemic and Other Adverse Events.....	73
8.4.2.1.3	Analysis of Unsolicited Adverse Events	73
8.4.2.1.4	Analysis of Safety Laboratory Values.....	74
8.4.2.2	Analysis of Primary Efficacy Objective(s).....	74
8.4.2.2.1	Statistical Hypotheses.....	74
8.4.2.2.2	Analysis Sets	74
8.4.2.2.3	Statistical Methods	75
8.4.2.3	Analysis of Primary Immunogenicity Objective(s).....	75
8.4.2.3.1	Statistical Hypotheses.....	75
8.4.2.3.2	Analysis Sets	75
8.4.2.3.3	Statistical Methods	75
8.4.3	Analysis of Secondary Objective(s)	75
8.4.3.1	Analysis of Secondary Safety Objective(s).....	75
8.4.3.1.1	Analysis of Extent of Exposure.....	75
8.4.3.1.2	Analysis of Solicited Local, Systemic and Other Adverse Events.....	75

8.4.3.1.3	Analysis of Unsolicited Adverse Events	75
8.4.3.1.4	Statistical Hypotheses	75
8.4.3.1.5	Analysis Sets	76
8.4.3.1.6	Statistical Methods	76
8.4.3.2	Analysis of Secondary Efficacy Objective(s)	76
8.4.3.2.1	Statistical Hypotheses	76
8.4.3.2.2	Analysis Sets	76
8.4.3.2.3	Statistical Methods	76
8.4.3.3	Analysis of Secondary Immunogenicity Objective(s)	76
8.4.3.3.1	Statistical Hypotheses	76
8.4.3.3.2	Analysis Sets	76
8.4.3.3.3	Statistical Methods	76
8.4.4	Analysis of Exploratory Objectives	77
8.4.4.1	Analysis of Exploratory Safety Objective(s)	77
8.4.4.2	Analysis of Exploratory Efficacy Objective(s)	77
8.4.4.3	Analysis of Exploratory Immunogenicity Objective(s)	77
8.5	Sample Size and Power Considerations of Primary Objectives	77
8.6	Interim Analysis	77
9.	SOURCE DOCUMENTATION, STUDY MONITORING AND AUDITING	78
9.1	Source Documentation	78
9.2	Study Monitoring, Auditing and Source Data Verification	79
10.	DATA MANAGEMENT	80
10.1	Data Entry and Management	80
10.2	Data Clarification	80
10.3	Data Protection	80
11.	RECORD RETENTION	81
12.	USE OF INFORMATION AND PUBLICATION	82
13.	ETHICAL CONSIDERATIONS	83
13.1	Regulatory and Ethical Compliance	83

13.2	Informed Consent Procedures.....	83
13.3	Responsibilities of the Investigator and IRB/EC.....	84
13.4	Protocol Amendments	85
14.	REFERENCE LIST	86

PROTOCOL SYNOPSIS H03_04TP

Name of Sponsor: Sclavo Behring Vaccines Institute for Global Health (SBVGH)	Protocol number: H03_04TP	Generic name of study vaccine(s): <i>S. sonnei</i> 1790GAHB Vaccine
Title of Study: A Phase 2a, Observer Blind, Randomized, Controlled, Single Center Study To Evaluate The Safety, Reactogenicity And Immunogenicity Of 2 Doses Of The SBVGH 1790GAHB Vaccine Against <i>Shigella Sonnei</i> , Administered Intramuscularly In Adult Subjects From A Country Endemic For Shigellosis.		
Study Period: Each subject will be followed up for approximately 1 month after last vaccination. Following the screening period, the total study duration will be approximately 2 months for each subject.		Clinical Phase: Phase 2a
Background and Rationale: Shigellosis remains a major health problem in developing countries with approximately 100 million cases per year mostly in children ≤ 5 years. Antibiotic resistance of <i>Shigella</i> is increasing and no vaccine is currently available against shigellosis. Among the <i>Shigella</i> serotypes that are epidemiologically more relevant, the investigators of the recently published GEMS study, aimed to identify the main causes of moderate to severe diarrhea (MSD) in Africa and Asia (Livio et al., 2014) have concluded that a quadrivalent vaccine containing <i>S. sonnei</i> and 3 serotype/subserotypes of <i>S. flexneri</i> (<i>S. flexneri</i> 2a, <i>S. flexneri</i> 3a, and <i>S. flexneri</i> 6) can provide broad coverage against <i>Shigella</i> serotypes (up to 65%), which cause shigellosis in the developing world, and can also provide broad coverage for travelers. The SBVGH candidate vaccine against shigellosis caused by <i>Shigella sonnei</i> (1790GAHB) has been developed with a novel technology based on high yield production of Generalized Modules for Membrane Antigens (GMMA) from <i>S. sonnei</i> . GMMA are outer membrane particles naturally released from the <i>S. sonnei</i> during		

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Sclavo Behring Vaccines Institute for Global Health (SBVGH)	H03_04TP	<i>S. sonnei</i> 1790GAHB Vaccine
<p>growth.</p> <p>The candidate <i>S. sonnei</i> 1790GAHB vaccine was chosen as a prototype Shigella vaccine based on the GMMA technology since <i>S. sonnei</i> is among the most common serotypes causing dysentery in humans.</p> <p>This SBVGH <i>S. sonnei</i> 1790GAHB vaccine has been tested in two parallel Phase 1 trials in European adult population: H03_01TP looking at the intramuscular (IM) administration and H03_02TP looking at the intradermal (ID), intranasal (IN) and IM administration. Based on the preliminary results from these phase 1 studies, it has been decided to proceed with further development using only the IM route of immunization and to test in the proposed H03_04TP study two doses selected based on the results from H03_01TP study.</p> <p>The two doses have been selected as follows: 25 µg is the lowest dose that in phase 1 consistently induced antibody titers comparable to antibodies in a population of convalescent subjects after natural infection while 100 µg is tested for two reasons i.e.1) a higher dosage may be needed to induce high antibody titers in endemic population and 2) there is a need to test in African populations the tolerability of a higher dose of GMMA that will be ultimately needed in the multivalent vaccine against Shigellosis.</p> <p>The purpose of H03_04TP study is to evaluate the safety and the immunogenicity of two different doses (25 µg and 100 µg) of the SBVGH <i>S. sonnei</i> 1790GAHB vaccine in healthy adults and represents the first step towards testing of the GMMA vaccine in the vaccine target population of children from developing countries where shigellosis is endemic.</p>		
<p>Study Objectives:</p> <p>Primary Objective:</p> <p>To evaluate the safety profile of two injections of <i>S. sonnei</i> 1790GAHB vaccine in healthy adults in a <i>Shigella</i> endemic country</p>		

Name of Sponsor: Sclavo Behring Vaccines Institute for Global Health (SBVGH)	Protocol number: H03_04TP	Generic name of study vaccine(s): <i>S. sonnei</i> 1790GAHB Vaccine										
Secondary Objective: To evaluate the immunogenicity profile of two injections of <i>S. sonnei</i> 1790GAHB vaccine in healthy adults by measuring the anti-LPS <i>S. sonnei</i> serum IgG.												
Study Design: This is a phase 2a, randomized, controlled, single center, observer blind trial that will enroll approximately 72 healthy adult subjects, 18-45 years of age inclusive. As currently there is no vaccine available against shigellosis, the safety of the 1790GAHB vaccine will be evaluated against two licensed control vaccines: one dose of Menveo as 1 st injection and one dose of Boostrix as 2 nd injection. Subjects will be randomized to one of the three parallel treatment arms in a 1:1:1 ratio to receive either the study vaccine dose A (25 µg), the study vaccine dose B (100 µg) or the control vaccines as follows: Table 1 Overview of the Study Groups <table border="1"><thead><tr><th></th><th>No. Subjects</th></tr></thead><tbody><tr><td>Group A (25 µg)</td><td>24</td></tr><tr><td>Group B (100 µg)</td><td>24</td></tr><tr><td>Group C (Control)</td><td>24</td></tr><tr><td>Total</td><td>72</td></tr></tbody></table> Two injections of the study vaccine or the two control vaccines will be administered 28 days apart. The study includes a screening visit (performed at study Days -28/-1), five Clinical Visits (performed at study Day 1, 7, and 28 days after 1 st injection and 7 and 28 days after 2 nd injection) and daily home visits performed for 6 days after the 1 st and 2 nd injection. During the screening period before 1 st vaccination, subjects providing informed consent				No. Subjects	Group A (25 µg)	24	Group B (100 µg)	24	Group C (Control)	24	Total	72
	No. Subjects											
Group A (25 µg)	24											
Group B (100 µg)	24											
Group C (Control)	24											
Total	72											

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<p>will be screened for general health status.</p> <p>Blood (approximately 10 mL) will be drawn from all subjects before the first injection (visit 1), 28 days after the first injection (visit 3), and 28 days after the second injection (visit 5) for immunogenicity evaluation.</p> <p>Appropriately trained study staff will be instructed to complete the subject's diary card during daily home visits following discussion with the subject to (i) describe solicited local (i.e. injection site erythema, induration and pain) and systemic (i.e. fever [temperature $\geq 38.0^{\circ}\text{C}$ measured axillary], fatigue, malaise, myalgia, chills, arthralgia and headache) adverse events (AEs) occurring during the day of each injection visit (visit 1 and 3) and for the following 6 days; (ii) indicate if any analgesic/antipyretic to prevent or treat pain/fever was taken after injection.</p> <p>In addition to the solicited adverse events data, from study visit 1 through visit 5 (i.e. study termination visit) any unsolicited AE, all serious adverse events (SAEs), all AEs leading to vaccine/study withdrawal, all Adverse Events of Special Interest (AESI, see below) and all concomitant medications associated with those events, will be collected and recorded in the subject's source document and on an Adverse Events CRF(s). In case of such events, subjects will be instructed to contact the site as soon as possible to report the event(s).</p> <p>Reactive arthritis will be collected and analyzed as an AESI for this study.</p>		
<p>Number of Subjects planned:</p> <p>A total of 24 subjects per group will be enrolled in each of the three study groups in order to have 20 evaluable subjects in each group. This allows for a drop-out rate of 15% during the course of the trial. Thus a total of approximately 72 subjects will be enrolled in the trial.</p>		
<p>Study Population and Subject Characteristics:</p> <p>Subjects, who meet all inclusion criteria and none of the exclusion criteria (refer to section 4. Selection Of Study Population), will be eligible for enrollment.</p>		

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Study Procedures: <p>Before any study procedure is performed, informed consent will be obtained and subjects will be enrolled only after their eligibility for participation is confirmed by the investigator.</p> <p>Study will be explained in either Swahili or in English where prospective participants express a preference. For subjects who are illiterate the whole informed consent document will be explained in the presence of an impartial witness.</p> <p>For all women of childbearing potential the screening will include the evaluation of human chorionic gonadotropin (hCG) in blood to exclude pregnancy and women refusing to perform the test or with positive test will be immediately excluded from the study. Informed consent process will include counseling about pregnancy and family planning. hCG in urine will then be evaluated (using a dipstick) at visit 1 before randomization; it will be repeated on visit 3 before 2nd vaccination and on the day of the last study visit (visit 5). The pregnancy test should be negative prior to each injection. All pregnancies that occur during the study will be recorded into the Pregnancy CRF appropriate pages and followed until delivery or abortion, as applicable.</p> <p>Female subjects of child-bearing potential must use acceptable birth control measures (defined as oral, injected or implantable contraceptives) for the two months preceding 1st vaccination and during entire study participation.</p> <p><u>Vaccination Procedure:</u> Subjects will receive the study vaccines at study Day 1 (visit 1) and at study Day 29 (visit 3) in accordance with group assignment in a blinded fashion. Vaccines should be administered by unblinded study personnel by deep IM injection into the deltoid area of the non-dominant arm unless there is a reason to vaccinate elsewhere which should be documented. After receiving the vaccination, subjects will be observed for at least 1 hour for any immediate reactions.</p> <p><u>Procedure for collection of solicited AE:</u> appropriately trained study staff will record solicited local and systemic adverse events and medications/vaccinations taken/received by the subject into a Diary Card and following discussion with the subject. Beginning in the evening following study vaccine administration (approximately 6 hours), and daily</p>		

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<p>thereafter through the following 6 days, solicited local and systemic adverse events including other reactions (i.e. body temperature measurements and use of analgesics/antipyretics) will be reported daily by field workers on a Diary Card. For this purpose daily home visits will be performed for 6 days after each injection and a consultation at the vaccination clinic will be performed at 7 days after each injection (clinic Visits 2 and 4).</p>		
<p>Any unsolicited AE occurring during the day of injection with study vaccines and for the following 28 days, solicited local and systemic AE that continue beyond 7 days after study vaccination, all SAEs, AEs leading to vaccine/ study withdrawal, AESI and concomitant medications associated with those events will be collected and recorded from Visit 1 to Visit 5 in the subject's records and on the Adverse Events CRF. The information obtained by study personnel will be recorded on the appropriate web based system, referred to as Electronic Data Capture (EDC).</p>		
<p><u>Safety procedures:</u> All subjects providing informed consent will undergo a general physical examination at the screening visit (study Day -28/-1) and at visit 1 (Day 1) for evaluation of the general health status by clinical assessment, verification of inclusion and exclusion criteria and review of medical history. On visit 3, prior to 2nd injection with the investigational vaccine or control, subjects will be assessed for continued eligibility with regard to the inclusion and exclusion criteria and physical examination. A brief symptom-directed physical examination (if necessary according to symptoms the subject has reported) will be performed at Clinic visits 2, 4 and 5.</p>		
<p><u>Blood Draw Procedure:</u> Approximately 10 mL of blood will be obtained as part of the initial screening for hematology, renal, and liver function tests, and serological tests for hepatitis B and HIV. In addition, an analysis of the urine will be performed. Each randomized subject will have 10 mL of blood drawn before the 1st and 2nd vaccination, and 28 days after the 2nd vaccination for immunological studies. Additional blood draws of 10 mL for hematology, renal and liver panels will be obtained 7 and 28 days after 1st and 2nd vaccination, urinalysis will be conducted at the same time points.</p>		
<p>All subjects will undergo study termination procedures on visit 5 (28 days after 2nd injection visit).</p>		

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<p>Study Vaccines:</p> <p>SBVGH <i>S. sonnei</i> (1790GAHB) vaccine</p> <p>The investigational agent is the SBVGH <i>S. sonnei</i> vaccine. The vaccine consists of <i>S. sonnei</i> 1790-GMMA (approximately 200 µg/mL, measured by protein content) adsorbed to Alhydrogel (0.7 mg Al³⁺/mL) in Tris-buffered saline. The vaccine does not contain any preservative and is available as a liquid formulation in single dose vials with 0.7 mL of injectable solution containing approximately 140 µg of GMMA (as protein content), adsorbed onto 0.49 mg Al³⁺.</p> <p>The vaccine will be used at two different antigen doses obtained by bed-side mixing. Following dilution with Alhydrogel (0.7 mg Al³⁺/mL) in Tris-buffered saline, the volume administered will be 0.5 mL for both doses:</p> <p>Group A: Each 0.5 mL dose of 1790GAHB will contain approximately 25 µg of GMMA total protein and 0.35 mg of Al³⁺.</p> <p>Group B: Each 0.5 mL dose of 1790GAHB will contain approximately 100 µg of GMMA total protein and 0.35 mg of Al³⁺.</p> <p>Control vaccines</p> <p>Menveo: vaccine indicated for active immunization to prevent invasive meningococcal disease caused by <i>Neisseria meningitidis</i> serogroups A, C, Y and W-135. The vaccine contains no preservative or adjuvant. Each 0.5 mL dose of vaccine contains 10 µg MenA oligosaccharide, 5 µg of each of MenC, MenY and MenW-135 oligosaccharides conjugated to 32.7 to 64.1 µg of CRM₁₉₇ protein.</p> <p>Boostrix: Vaccine indicated for active booster immunization against tetanus, diphtheria, and pertussis. It contains tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis antigens (inactivated pertussis toxin [PT], formaldehyde-treated filamentous hemagglutinin [FHA] and pertactin). Each antigen is individually adsorbed onto aluminum hydroxide.</p>		

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No other concomitant vaccines or treatments will be used as part of study procedures.		
<p>Primary Endpoint(s):</p> <p>The safety profile of the two injections of the investigational vaccine and control(s) will be assessed through measurement of the following:</p> <ol style="list-style-type: none"> Number and percentage of subjects with solicited local and systemic adverse reactions during 7 days following each vaccination. Numbers and percentage of subjects with deviations from normal ranges of safety laboratory data after each vaccination. Number and percentage of subjects with reported unsolicited adverse events during 28 days following each vaccination. Number and percentage of subjects with reported SAEs throughout the study duration. Number and percentage of subjects with reported reactive arthritis (AESIs). <p>Secondary Endpoint(s):</p> <p>The measures of the immunogenicity outcome, (i.e., the anti-LPS <i>S. sonnei</i> serum IgG), will include:</p> <ol style="list-style-type: none"> IgG geometric mean concentrations (GMCs) pre-vaccination (Day 1), 28 days after 1st vaccination and 28 days after 2nd vaccination, as determined by Enzyme-linked Immunosorbent Assay (ELISA), and applicable geometric mean ratios between post- and pre-vaccination samples. Number and percentage of subjects with seroresponse for anti- LPS <i>S. sonnei</i> at 28 days after 1st vaccination and 28 days after 2nd vaccination <p>Seroresponse is aimed to define a significant increase in post vaccination samples based on the biological performance of this specific serology assay and it is defined as:</p> <ul style="list-style-type: none"> - If the baseline value is greater than 50 ELISA Units (EU) then an increase of at 		

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<p>least 50% in the post-vaccination sample as compared to baseline [i.e. ((Post-vac minus baseline)/baseline)100% \geq 50%]</p> <p>- If the baseline value is less or equal to 50 EU then an increase of at least 25 EU in the post-vaccination sample as compared to baseline [i.e. (post-vac minus baseline) \geq 25 EU]</p> <p>c. Number and percentage of subjects with titers post vaccination concentration \geq 121 for anti-LPS <i>S. sonnei</i> at 28 days after 1st vaccination and 28 days after 2nd vaccination</p> <p>d. IgG GMCs pre-vaccination (Day 1), 28 days after 1st vaccination and 28 days after 2nd vaccination by antibody titer at baseline (i.e., above vs. below the assay detection limit), as determined by ELISA, and applicable geometric mean ratios between post- and pre-vaccination samples.</p> <p>e. Number and percentage of subjects with seroresponse for anti- LPS <i>S. sonnei</i> at 28 days after 1st vaccination and 28 days after 2nd vaccination by antibody titer at baseline (i.e., above vs. below the assay detection limit).</p> <p>A post vaccination concentration \geq 121 anti-LPS serum IgG units in the SBVGH ELISA corresponds to a titer of 1:800 in the ELISA method used by Cohen et al. (1989 J. Clin. Microbiol. 27:162). This antibody level is the median antibody concentration of a set of 87 convalescent subjects previously infected by <i>S. sonnei</i>. The value of 121 anti-LPS serum IgG units in the SBVGH ELISA was determined by calibration against the Cohen ELISA (i.e., the SBVGH standard serum was tested in Cohen's lab using the Cohen's methodology).</p>		
Exploratory endpoints		
Other assays, including serum secretory IgA, might be done to further characterize the immune response to the study vaccine.		
Statistical Analyses:		
This Phase 2a safety and immunogenicity study is aimed to descriptively evaluate the safety and immunogenicity profiles of the study vaccines. No specific hypotheses are		

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<p>tested in this trial. A total of 72 subjects will be enrolled in this study: 24 subjects per group will be enrolled in order to have 20 evaluable subjects in each group (15% drop-out rate). With 20 evaluable subjects in each vaccine group, the probability of observing at least one adverse event per group is 87%, if the actual rate of the event is 10%.</p>		
<p>Interim Analysis:</p> <p>No interim analysis will be performed.</p>		
<p>Data Monitoring Committee:</p> <p>An independent data safety monitoring board (DSMB) will receive a summary of all safety data (solicited local and systemic AE, unsolicited AE and SAEs) and listings of clinically significant modifications in hematology, blood chemistry and urine dipstick/urinalysis test values obtained during one week follow-up post-first vaccination of the initial 18 subjects. Further recruitment will be put on hold until the DSMB opinion is received.</p> <p>Throughout the entire study the DSMB will be consulted for any safety issue that might be reported during the trial.</p> <p>See section 3.8 of the protocol for more details.</p>		

Table 2 Time and Events Table

Study Periods		Screening	Treatment						
Visit Type		Clinic	Clinic	Home visits	Clinic	Clinic	Home visits	Clinic	Clinic
Visit Number		n/a	1	Daily for 6 days	2	3	Daily for 6 days	4	5
Study Day*		n/a	1		V1+7	V1+28		V3+7	V3+28
Time Window (days) (min/max)		-28/-1	0	0	0/+1	0/+7	0	0/+1	0/+7
Study Event	References								
Study Treatment									
Vaccination	Section 5.2		X			X			
Screening and Safety									
Informed Consent ^[a]	Section 5.1.1	X							
Medical History	Section 5.1.2	X							
Physical Examination ^[b]	Sections 5.1.2 and 5.3.1	X	X		X	X		X	X
Pregnancy Test	Sections 5.5.2 and 5.1.2	X (blood)	X (urine)			X (urine)			X (urine)
Inclusion/Exclusion Criteria ^[c]	Section 4.0	X	X			X			
Randomization	Section 5.1.4		X						
Blood draw for Serology for HIV, HBV	Section 5.1.2	X							
Safety blood draw ^[d]	Sections 3.5 and 5.1.2	X			X	X		X	X
Urine dipstick (urinalysis as required)	Sections 3.5 and 5.1.2	X			X	X		X	X
Post Injection Assessment ^[e]	Sections 5.1.2		X			X			

Study Periods		Screening	Treatment						
Visit Type		Clinic	Clinic	Home visits	Clinic	Clinic	Home visits	Clinic	Clinic
Visit Number		n/a	1	Daily for 6 days	2	3	Daily for 6 days	4	5
Study Day*		n/a	1		V1+7	V1+28		V3+7	V3+28
Time Window (days) (min/max)		-28/-1	0	0	0/+1	0/+7	0	0/+1	0/+7
Study Event	References								
Post Injection Assessment ^[f]	Sections 5.2.1 and 5.3.1 and 5.3.2		X	X	X	X	X	X	
Assess all AEs ^[g]	Section 7.1		X	X	X	X	X	X	X
Assess/ Inquire about AE leading to withdrawal, all SAE and AESI ^[h]	Sections 7.1.4.1 and 7.1.3		X	X	X	X	X	X	X
Assess Medications and Vaccinations	Sections 5.1.2 and 6.5	X	X	X	X	X	X	X	X
Study Termination	Section 5.5								X
Immunogenicity									
Serology blood draw	Sections 3.5 and 7.3		X			X			X

* Study days should be calculated based on the actual date of the previous visit (as to comply with requested Study Visit Time Window)

- Informed Consent to be obtained before any study procedure
- Physical examination must be performed by a qualified health professional in accordance with local regulations and licensing requirements designated within the Site Responsibility Delegation Log.
- Compliance with Exclusion/Inclusion criteria should be verified.
- In case of neutropenia, Complete Blood Count to be repeated on a weekly basis until resolution. If neutropenia occurs at the last study visit, Complete Blood Count to be repeated on a regular basis until resolution.
- A post-injection local and system adverse event and body temperature measurement will be performed approximately 30 and 60 minutes after each vaccination during the clinic visit.

- f. Beginning in the evening following study vaccine administration (approximately 6 hours), and daily thereafter through the following 6 days, solicited local and systemic adverse events including body temperature measurements and use of analgesics/antipyretics will be reported by field workers in a diary card based on subject's observation and interview.
- g. All unsolicited adverse events will be captured through 28 days following each vaccination.
- h. SAEs, AESI and AEs leading to study or vaccine withdrawal will be collected through entire study duration.

Table 3 Safety Tests Table

HEMATOLOGY	
White Blood Cells (WBC)	
Red Blood Cells (RBC)	
Haemoglobin	
Haematocrit	
Platelets	
Eosinophils	
Basophils	
Neutrophils	
Monocytes	
Lymphocytes	
Prothrombin time*	
CLINICAL CHEMISTRY	
Total bilirubin	
Aspartic Aminotransferase (ASAT/GOT)	
Alanine Aminotransferase (ALAT/GPT)	
γ -Glutamyl Transferase (γ -GT)	
Lactic Dehydrogenase (LDH)	
Alkaline Phosphatase (AP)	
Total Proteins*	
Glucose (random glucose)	
Blood Urea Nitrogen (BUN)	
Creatinine	
Sodium	
Potassium	
SEROLOGY for VIROLOGY	
HbsAg*	
HIV antibodies*	
PREGNANCY TEST	
Human chorionic gonadotropin (hCG) in blood *	
Human chorionic gonadotropin (hCG) in urine	

* Performed at screening only

URINE DIPSTICK	
Glucose	
Proteins	
pH	
Ketones	
Nitrites	
Blood	
URINALYSIS: Microscopic test on urine (performed if urine dipstick shows deviations from normal values)	
Leucocytes (WBC)	
Erythrocytes (RBC)	
Epithelial Cells	
Casts	
Bacteria	

LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Events of Special Interest
AP	(Statistical) Analysis Plan
ANC	Absolute Neutrophil Count
BMI	Body mass index
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CI	Confidence Interval
CRF	Case Report Form
CRO	Contract Research Organization
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EDT	Electronic Data Transfer
ELISA	Enzyme-linked Immunosorbent Assay
EMA	European Medicines Agency
FAS	Full Analysis Set
FDA	Food and Drug Administration
GCP	Good Clinical Practices
GMC	Geometric Mean Concentration
GMMA	Generalized Modules for Membrane Antigens
GMR	Geometric Mean Ratio
GMT	Geometric Mean Titer
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ID	Intradermal
IM	Intramuscular
IN	Intranasal
IV	Intravenous
IRB	Institutional Review Board
ITT	Intention-To-Treat
MITT	Modified Intention-To-Treat
MSD	Moderate to Severe Diarrhea
LPS	Lipopolysaccharide
LSLV	Last Subject Last Visit

MedDRA	Medical Dictionary for Regulatory Activities
NOCD	New onsets of chronic diseases
OAg	O antigen (of <i>Shigella sonnei</i>)
OMV	Outer Membrane Vesicles
PO	Per Os (orally)
PP	Per Protocol
PSB	Product Stewardship Board
ReA	Reactive arthritis
SAE	Serious Adverse Event
SBVGH	Sclavo Behring Vaccines Institute for Global Health
SDA	Source Data Agreement
SOC	System Organ Class
SOP	Standard Operating Procedure
SPC	Summary of Product Characteristics
WHO	World Health Organization

1. BACKGROUND AND RATIONALE

Shigella spp. are Gram-negative bacteria that infect the intestinal epithelium and are major causes of diarrhea, including dysentery. *Shigella* is transmitted by the fecal-oral route and taken up by contaminated food or water. It is endemic throughout the world but the main burden of disease is in developing countries. In 2009, the World Health Organization (WHO) estimated approximately 125 million cases of shigellosis per year in Asia alone (Bardhan et al., 2010). Ninety-nine percent of all cases occur in developing countries and approximately 70% in children younger than 5 years of age (Kotloff et al., 1999). Current estimates of mortality vary between 108,000 worldwide (http://www.who.int/vaccine_research/diseases/diarrhoeal/en/index6.html) and 14,000 in Asia where previously 80% of all deaths were estimated to occur. Sixteen serotypes (all 14 *S. flexneri*, *S. sonnei*, and *S. dysenteriae* type I) are considered to be of global importance (Zhang et al., 2011) with *Shigella sonnei* being the most common serotype worldwide.

Among the *Shigella* serotypes that are epidemiologically more relevant, the investigators of the recently published GEMS study, aimed to identify the main causes of moderate to severe diarrhea (MSD) in Africa and Asia (Livio et al., 2014) have shown that 8 *Shigella* serotypes are approximately responsible for 87% of the disease. However, they concluded that a quadrivalent vaccine containing *S. sonnei* and 3 serotype/subserotypes of *S. flexneri* (*S. flexneri* 2a, *S. flexneri* 3a, and *S. flexneri* 6) can provide a sufficiently broad coverage against *Shigella* serotypes (up to 65%), which cause the majority of endemic pediatric shigellosis in the developing world, and can also provide broad coverage for travelers.

In target populations, treatment options for shigellosis are limited. Shigellosis can be treated with appropriate antibiotics. However, antibiotic resistance is increasing and many *Shigella* isolates are resistant to two or more of the common antibiotics ampicillin, chloramphenicol, nalidixic acid, co-trimoxazole. Resistance to third generation antibiotics, especially ciprofloxacin, has been reported to be emerging (Zhang et al., 2011). Still effective antibiotics include ceftriaxone that is administered intramuscularly or intravenously and is not easily accessible for people in impoverished communities.

No vaccine is available. Natural infection (experimental infection or vaccination with attenuated *Shigella*), leads to good protective immunity, but despite the generally high genetic conservation between serotypes, the protection is highly specific for the infecting serotype. This suggests that the dominant protective antigen is the O antigen (OAg) of the lipopolysaccharide (LPS). There have been many attempts to make a *Shigella* vaccine, using inactivated whole cell bacteria either orally or parentally (low efficacy, high reactogenicity for parenteral), attenuated live oral vaccines (no vaccine has yet obtained a useful balance between attenuation and efficacy), recombinant surface proteins (several projects at an early stage), O antigen conjugates (Levine et al., 2007). The latter have been the most successful to date with a parenteral *S. sonnei* and *S. flexneri* type 2OAg

conjugates tested in field trials in Israel that achieved 74% efficacy in adults (Cohen et al., 1997) with one immunization and 71% efficacy in children 3 years of age and older with 2 vaccinations (Passwell et al., 2003, Passwell et al., 2010). No significant efficacy was achieved in younger children in accordance with a very low immunogenicity in the young children. As expected for an OAg vaccine, this was highly serotype specific and the *S. sonnei* OAg afforded no protection against infection with *S. flexneri* nor did a *S. flexneri* 2a based OAg conjugate protect against *S. sonnei*. In addition, to achieve broad-spectrum protection against the 16 serotypes that are currently considered to be globally important, a multivalent OAg-vaccine will be needed. Therefore, new vaccine development approaches are needed.

Sclavo Behring Vaccines Institute for Global Health (SBVGH) candidate vaccine against shigellosis caused by *Shigella sonnei* (1790GAHB) is based on a parenteral vaccine targeting the OAg but using a new platform technology called Generalized Modules for Membrane Antigens (GMMA) as a novel delivery system which may be applicable also for other vaccines against Gram-negative pathogens. GMMA are naturally shed from the surface of Gram-negative bacteria and consist of outer membrane proteins, outer membrane lipids, including phospholipids and LPS, and enclosed periplasmic proteins (Beveridge, 1999). In the previous literature, GMMA are called outer membrane vesicles (OMV). However, this term has also been used for vesicles derived by detergent-extraction of homogenized bacteria which are used as vaccines, e.g. to control *Neisseria meningitidis* type B infections in New Zealand (MenNZB) or other broader coverage MenB vaccines (Bexsero). In order to clearly differentiate these different types of particles, the name 'GMMA' was introduced for the blebs spontaneously released from the cell surface (Berlanda Scorza et al., 2012). The natural arrangement of the outer membrane is preserved during the release of GMMA and therefore GMMA allow an optimal exposure of the antigens of the outer membrane for recognition by the host immune system. SBVGH has developed an economic process to purify GMMA in large quantities from high density cultures of bacteria genetically modified to increase GMMA production and generate a LPS with low endotoxicity, suitable for use in humans. *S. sonnei* was chosen as the proof of concept for the GMMA technology and as a prototype *Shigella* GMMA vaccine since *S. sonnei* is among the most common serotypes causing dysentery in humans.

A comprehensive review of SBVGH 1790GAHB vaccine is contained in the Investigator's Brochure (IB) supplied by SBVGH; this document should be reviewed prior to initiating the study.

1.1 Background

This SBVGH *S. sonnei* 1790GAHB vaccine has been tested in two Phase 1 trials in European adult population: H03_01TP, looking at the intramuscular (IM) administration,

and H03_02TP, looking at the intradermal (ID), intranasal (IN) and IM administration. Based on the results from these phase 1 studies, it has been decided to proceed with further development using only the IM route of immunization and to test in the proposed H03_04TP study two doses selected based on the results from H03_01TP study. The two doses have been selected as follows: 25 µg is the lowest dose that in phase 1 induced antibody titers comparable to antibodies in a population of convalescent subjects after natural infection already after the first vaccination, while 100 µg is tested for two reasons i.e. 1) a higher dose may be needed to induce high antibody titers in endemic population and 2) there is a need to test in African populations the tolerability of a higher dose of GMMA that will be ultimately needed in the multivalent vaccine against Shigellosis.

The proposed H03_04TP trial is the logical continuation of the two Phase 1 studies and is aimed to evaluate the safety and immunogenicity profile of 1790GAHB in adults from an African country, where shigellosis remains a major health problem.

The dose regimen tested in phase 1 studies (three doses given 1 month apart) did not show any significant benefit from the third dose in terms of immunogenicity, therefore a two dose schedule was selected for the current trial. The two dose schedule will allow evaluation of the immunogenicity in the endemic population where one dose may be not enough to induce the same immunogenicity observed in European population.

During the two phase 1 trials three subjects of African descent experienced a transient and clinically asymptomatic decrease of circulating neutrophils (two episodes were graded as severe and one as moderate) that was finally classified as "benign ethnic neutropenia". Although this finding was not associated with any clinical illness, some precautionary procedures for enrollment and monitoring of trial subjects have been introduced to protect the safety of study subjects (refer to [Section 3.7, Procedures Related to Neutropenia](#)).

The trial will be conducted in compliance with the protocol, GCP and applicable regulatory requirement(s).

1.2 Rationale

The purpose of H03_04TP study is to evaluate the safety and the immunogenicity of two different doses (25 µg and 100 µg) of the SBVGH *S. sonnei* 1790GAHB vaccine in healthy adults in Africa and represents the first step towards testing of the GMMA vaccine in the vaccine target population of children from developing countries where Shigellosis is endemic.

2. OBJECTIVES

2.1 Primary Objective(s)

Primary Safety Objective(s)

To evaluate the safety profile of two injections of *S. sonnei* 1790GAHB vaccine in healthy adults in a Shigella endemic country

2.2 Secondary Objective(s)

Secondary Immunogenicity Objective(s)

To evaluate the immunogenicity profile of two injections of *S. sonnei* 1790GAHB vaccine in healthy adults at 28 days after 1st vaccination and 28 days after 2nd vaccination, by measuring the anti-LPS *S. sonnei* serum IgG.

2.3 Exploratory Objective(s)

This study has no exploratory objectives.

3. STUDY DESIGN

3.1 Overview of Study Design

This is a phase 2a, randomized, observer blind, controlled single center study in healthy adult women and men healthy volunteers evaluating the safety and immunogenicity of two doses of the SBVGH vaccine against *Shigella sonnei* infections (1790GAHB). As no vaccine is currently available against shigellosis, the safety profile of the 1790GAHB vaccine will be evaluated in comparison to that of two control vaccines, Menveo as 1st vaccination and Boostrix as 2nd vaccination. All subjects and blinded study personnel will be blinded to treatment throughout the study.

This clinical trial has been designed to minimize pain, discomfort, fear and any other foreseeable risks. During the screening period, subjects giving informed consent will be screened for general health status. No pharmacokinetic tests will be performed as evaluation of pharmacokinetic properties is not required for vaccines unless new delivery systems are employed or when the vaccine contains novel adjuvants or excipients (Berlanda Scorza et al., 2012). Subjects who meet all inclusion criteria and none of the exclusion criteria, with screening tests within normal values and women of child bearing potential with negative pregnancy test will be eligible for enrollment. Female subjects of child bearing potential must use birth control measures during study participation.

A total of 72 eligible subjects will be assigned to one of the three parallel treatment arms in a 1:1:1 ratio to receive either the study vaccine dose A (25 µg), the study vaccine dose B (100 µg) or the control vaccines as follows:

Table 3.1-1 Overview of the Study Groups

	No. Subjects
Group A (25 µg)	24
Group B (100 µg)	24
Group C (Control)	24
Total	72

Within each group, in an observer-blind fashion, subjects will be randomized to receive two intramuscular vaccinations, four weeks apart.

Screening/baseline clinical safety labs will take place before Visit 1 (from -28 up to -1 day). Blood will be obtained as part of the initial screening for hematology, renal, and liver function tests, and serological tests for hepatitis B and HIV. In addition, an analysis of the urine will be performed. Each randomized subject will have blood collected before the 1st and 2nd vaccination, and 28 days after the 2nd vaccination for immunological

testing. Additional blood draws for hematology, renal and liver panels will be obtained 7 and 28 days after 1st and 2nd vaccination, urine dipstick/urinalysis will be conducted at the same time points. All individuals with a neutropenia occurring at any time during the study will have additional blood draws to repeat complete blood count on a weekly basis until the neutropenia resolves (for classification of neutropenia during the trial, refer to [Section 3.7, Procedures Related to Neutropenia](#)).

Subjects will be observed at the clinic for at least 1 hour after each vaccination. In order to avoid excluding participants who cannot read and write, safety information (solicited local and systemic AE including body temperature measurements and use of analgesics/antipyretics) and medications/ vaccinations received beginning in the evening following study vaccine administration and daily thereafter through the following 6 days, will be recorded by appropriately trained study staff in a Diary Card, following discussion with the subject. For this purpose daily home visits will be performed on the day of the injection and for 6 days after each injection and a consultation at the vaccination clinic will be performed at 7 days after each injection (clinic Visits 2 and 4).

Any unsolicited AE, serious adverse events (SAEs), all AEs leading to vaccine/study withdrawal, and reactive arthritis (adverse event of special interest (AESI)) and all concomitant medications associated with those events, will be collected for the entire study and recorded in the subject's source document.

3.2 Study Period

Each subject should expect to participate in the study for 2 months, from the time of enrolment through the last study visit.

3.3 Blinding Procedures

The identity of the study vaccine and control cannot be concealed as presentations are different.

Therefore an observer blind study design has been chosen: during the study, designated unblinded trained and qualified site staff (please see [section 5.3](#)) will be responsible for preparing the study vaccine or controls out of view of the subject and an unblinded nurse(s) or clinician(s) will be responsible for administering the study vaccines to the subjects. The unblinded staff will be instructed not to reveal the identity of the study vaccines either to the subject or the other investigative site personnel involved in the conduct of the trial. The designated unblinded pharmacists, nurse(s) or clinician(s) will not take part in evaluating the subject(s) for safety or collect study data after the administration of the study vaccine.

Study vaccines allocations will not be available to the investigator or blinded personnel monitoring the trial until after the completion of the trial and final data review. Adherence to the randomization list will be verified by a designated and unblinded Study Monitor, independent of the staff involved in the regular monitoring of the study, by checking the randomization list against the vaccination records maintained at the study site.

Except in the case of medical necessity, a subject's treatment should not be unblinded without the approval of the Sponsor. In such instance of medical emergency, every effort should be made to contact the Sponsor prior to unblinding. If unblinding should occur (by either accidental unblinding or emergency unblinding for a serious adverse event) prior to completion of the study, the investigator must promptly contact the Sponsor and document the circumstances on the appropriate forms. Instructions regarding emergency unblinding will be provided to the investigator.

3.4 Data Collection

3.4.1 Data Collected from Subjects

The following data will be collected from each subject over the duration of their study participation:

- Demographic Information.
- Post-vaccination immediate reactions.
- Body temperature.
- Adverse Events.
- Medical History.
- Concomitant Medications.

All data collected must only be identified using the SBVGH Subject ID and Subject code, as described in [section 5.1.4, Randomization](#).

3.4.2 Tools Used for Data Collection

Data will be recorded in a Subject Diary Card by appropriately trained study staff and entered on Case Report Forms (CRFs).

The subject Diary Card will be the only source document allowed for solicited local and systemic adverse events (including body temperature measurements), starting after the initial 60 minute post-vaccination period at the clinic. The following additional rules apply to documentation of safety information collected in the Diary Card.

The Investigator or delegated staff should monitor the Diary Card status throughout the study for compliance and any solicited local and systemic adverse events that were of concern to the subject.

1. No corrections or additions to the information recorded by the trained study staff within the Diary Card will be allowed after it is delivered to the site.
2. Any blank or illegible fields on the Diary Card must be described as missing in the CRF.

Case Report Forms

This study utilizes Case Report Forms (CRFs) to collect study-related data from each subject. A qualified site staff member(s) is required to enter subject data in the CRFs in English based on the medical information available in each subject's source record.

Data should be entered into the CRF in a timely fashion following each subject's clinic visit, study procedure, or home visit. Each subject's CRF casebook will be compared with the subject's source records by a SBVGH-approved study monitor (or designee) over the duration of the study in order to ensure data collection accuracy.

3.5 Collection of Clinical Specimens

The following clinical specimens are required to be collected from each subject in this study:

- Blood
- Urine

Blood Specimens

A maximum of 10 mL of blood will be obtained as part of the initial screening. The blood will be used for pregnancy testing in females of child bearing potential and for hematology, renal and liver function tests, and serological tests for hepatitis B and HIV in all subjects as described in [synopsis Table 3, Hematological, Haematochemical Blood Tests and Urinalysis Table](#). The safety laboratory assays will be conducted at the Clinical Trials Laboratory, Kilifi, Kenya.

Each randomized subject will have blood collected before the 1st and 2nd vaccination, and 28 days after the 2nd vaccination for immunological assays. The blood volume will not exceed 10 mL at each time point in order to provide the necessary serum volume (approximately half of the blood draw volume) for the serology assays.

Serum samples will be stored frozen below -20°C. Shipment to the laboratories for analysis will be performed according to sites guidelines provided by the sponsor. The serologic assays will be conducted at the GSK Clinical Serology Laboratory, Marburg, (Germany) or a delegated laboratory.

Complete instructions for processing, labeling, storage and shipping of samples are included in the Clinical Specimen Laboratory Manual provided by sponsor and available in the Investigator Site File.

Additional blood draws of 10 mL for hematology, renal and liver function tests will be obtained 7 and 28 days after 1st and 2nd vaccination.

See [section 7, Assessments](#) for additional details.

The total amount of blood collected over the study period (including the screening) per subject will be 80 mL.

Blood samples must be collected in the appropriate manner, using exclusively materials and guidelines supplied by the sponsor. The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement.

The results of safety testing will be recorded in the source document and CRF.

Urine Specimens

Urine will be collected for urine dipstick/urinalysis as part of the initial screening, before the 2nd vaccination, and 7 and 28 days after the 1st and 2nd vaccination. Urine samples will be also used for pregnancy testing in females of child bearing potential before the 1st and 2nd vaccination, and 28 days after the 2nd vaccination.

The results of urine dipstick/urinalysis will be recorded in the source document and CRF.

3.6 Stopping/Pausing Guidelines

At the end of the 7 days observation period following 1st vaccination of the initial 18 subjects, a summary of all safety data (solicited local and systemic AE, unsolicited AE and SAE), listings of hematology, blood chemistry and urine dipstick/ urinalysis test values and respective clinically significant modifications, if any, will be provided to the DSMB. Based on evaluation of the safety data, the DSMB will make a recommendation, as to whether the enrollment can be completed or not. Additionally, DSMB will make a recommendation, as to whether the enrollment of further subjects could be done using the published local normal ranges for neutrophils in Africa (Karita et al., 2009) or should instead be continued using the western normal ranges adopted for the first 18 subjects.

Enrollment for the subsequent subjects will be started immediately after DSMB review and confirmation in writing.

During the course of the study, the occurrence of two cases of marked neutropenia (i.e. $< 0.5 \times 10^9/L$), or of febrile neutropenia (i.e., $ANC < 1.0 \times 10^9/L$ associated with fever) will result in study hold, unblinding of data by DSMB (but not by the investigators in the site), discussion of results with safety management team (i.e., Pharmacovigilance) and final decision made in consultation with DSMB, IRB and PI. For additional information, please refer to [Section 3.7, Procedures Related To Neutropenia](#)

Independent of the DSMB, SBVGH, as a sponsor, retains the right to halt the study at any time if there is a safety concern. If the study is prematurely terminated, the sponsor will promptly inform Regulatory Authorities and Ethic Committees on the decision of stopping the trial and no further enrollment or study immunizations will occur until written authorization is provided by the sponsor in conjunction with a recommendation to proceed by the DSMB and in consultation with Regulatory Authorities and Ethic Committees, as appropriate.

3.7 Procedures related to neutropenia

During the two phase 1 trials with 1790GAHB, three subjects of African descent experienced a transient and clinically asymptomatic decrease of circulating neutrophils (two graded as severe and one as moderate) that was finally classified as "benign ethnic neutropenia". Although this finding was not associated with any clinical illness, some precautionary procedures for enrollment and monitoring of trial subjects were introduced to protect the safety of study subjects, including selection of subjects with baseline ANC above $1.8 \times 10^9/L$.

In order to maintain a prudent approach, but, at the same time, to not compromise the scientific validity of the clinical trial by selecting participants that are not entirely representative of the local healthy population (if non-local laboratory reference intervals are used) a step-wise approach will be adopted for the present trial H03_04TP. More specifically, same as in Phase 1 trials, the more conservative western normal ranges will be adopted for ANC collected up to 7 days after 1st vaccination of the initial 18 subjects. .

Subsequently, all safety data from these 18 subjects will be evaluated by the DSMB which will make a recommendation as to whether the enrollment and assessment of neutropenia during the study of further subjects could be done using the local normal ranges published for neutrophils (Karita et al., 2009) and for which a consensus interval of 1.0 to $5.3 \times 10^9/L$ has been reached, or should be continued using the western normal ranges adopted for the first 18 subjects. This is also in consideration of the clinically benign nature of the events reported in the phase 1 studies and of the documented lower

absolute neutrophils counts in healthy African populations (one of the key targets for this vaccine) compared to western populations (Badenhorst et al., 1995, Ezeilo, 1972, Lugada et al., 2004),

Therefore, study procedures will be as follows:

- First subset of 18 subjects (prior to DSMB evaluation of data):

Subjects with ANC less than $1.8 \times 10^9/L$ at screening will not be enrolled in the study. All individuals with ANC $< 1.8 \times 10^9/L$, occurring at 7 days after the first dose, will have additional blood draws for complete blood count to be repeated on a weekly basis until the neutropenia resolves (ANC $\geq 1.8 \times 10^9/L$). In case the ANC is less than $0.5 \times 10^9/L$ after 1st vaccination, the subject will be immediately discontinued from 2nd vaccination and will have the test repeated on a weekly basis until the neutropenia resolves.

- All subjects (after DSMB evaluation of safety data from the initial 18 subjects with recommendation to switch to local normal ranges published for neutrophils):

Subjects with neutropenia defined as ANC less than $1.0 \times 10^9/L$ at screening will not be enrolled in the study. All individuals with a neutropenia (ANC $< 1.0 \times 10^9/L$), occurring at any time during the study 7 days after the first dose, will have additional blood draws for complete blood count to be repeated on a weekly basis until the neutropenia resolves (ANC $\geq 1.0 \times 10^9/L$). In case the ANC is less than $0.5 \times 10^9/L$ after 1st vaccination, the subject will be immediately discontinued from 2nd vaccination and will have the test repeated on a weekly basis until the neutropenia resolves.

All individuals with neutropenia either ongoing or with onset at visit 5 (or last study visit) will have additional blood draws for complete blood count to be repeated on a regular basis until the neutropenia resolves (ANC $\geq 1.0 \times 10^9/L$ or ANC $\geq 1.8 \times 10^9/L$, depending on recommendations given by DSMB after evaluation of safety data from the initial 18 subjects).

3.8 Data Monitoring Committee

A DSMB will be in place to receive from SBVGH a summary of all safety data (solicited local and systemic AEs, unsolicited AEs and SAEs) and listings of clinically significant modifications in hematology, blood chemistry and urine dipstick/ urinalysis test values obtained during one week follow-up post-first vaccination of the initial 18 subjects. Based on evaluation of the safety data, the DSMB will make a recommendation to SBVGH, as to whether the enrollment can be completed or not. Additionally, DSMB will make a recommendation to SBVGH, as to whether the enrollment of further subjects could be done using the published local normal ranges for neutrophils in Africa (Karita et al.,

2009) or should instead be continued using the western normal ranges adopted for the first 18 subjects.

In addition to the evaluation of the safety data from the initial 18 subjects, DSMB will be consulted for any safety issue that might be reported during the trial.

The composition of DSMB and the details of all relevant procedures will be documented in the DSMB Charter.

3.9 Premature Withdrawal from Study

Subjects may withdraw at any time, or be dropped from the study at the discretion of the investigator should any untoward effects occur and/or for safety reasons. In addition, a subject may be withdrawn by the investigator or the Sponsor if he/she violates the study plan or for administrative reasons. The investigator or study coordinator must notify the Sponsor immediately when a subject has been withdrawn due to an adverse event.

The circumstances above are referred to as premature withdrawal from the study, and the reason for premature withdrawal should be clearly documented and detailed in the source documentation. The investigator should make every attempt to evaluate the subject's safety, including resolution of ongoing AEs, at the time of premature withdrawal. If a subject wants to withdraw from the study before all doses are administered or prior to the last planned study visit, the subject will be asked to be followed for safety for the duration of the study. When a subject withdraws, or is withdrawn, from the study, the procedures described in [section 5.5.1, Early Termination Visit](#) should be completed if possible.

The reasons for premature withdrawal from the study include: Adverse event, death, withdrawal of consent, lost to follow-up, administrative reason, and protocol deviation. These reasons are described in greater detail below.

Adverse Event

For any subject withdrawn from study participation prior to the planned Study Termination Visit, it is important to determine if an AE was associated with the reason for discontinuing the study. This AE must be identified on the AE CRF page by indicating "Withdrawn from study due to AE". Any ongoing AEs at the time of study withdrawal must be followed until resolution or stabilization.

Subjects who develop a serious adverse event (SAE) judged to be possibly or probably related to the study vaccine, including hypersensitivity reactions, should not receive subsequent vaccination.

Death

For any subject withdrawn from study participation due to death, this should be noted on the Study Termination CRF page and the associated SAE that led to the death must be reported.

Withdrawal of consent

The subject can withdraw consent for participation in the study at any time without penalty or loss of benefit to which the subject is otherwise entitled. Reason for early termination should be deemed as “withdrawal of consent” if the subject withdraws from participation due to a non-medical reason (i.e., reason other than AE). If the subject intends to withdraw consent from the study, the investigator should clarify if the subject will withdraw completely from the study or if the subject will continue study participation for safety, or a subset of other study procedures. If the subject requests complete withdrawal from the study, no further study interventions will be performed with the subject.

Lost to Follow-Up

For subjects who fail to show up for final visits (clinic or home visits), or for three consecutive visits, study staff are encouraged to make at least three documented attempts to contact the subject to encourage the completion of study termination procedures. These efforts to contact the subject should be recorded in the source document. The termination date for the subject to be captured on the Study Termination CRF page is the date of the last successful contact (clinic visit or home visits) with the subject.

Administrative Reason

Examples for subjects withdrawn from the study due to administrative reason can include: Sponsor decision to terminate the study, subject meeting a pre-specified withdrawal criterion, subject discontinuation for insurance issues, moving, no time, etc. This reason should be noted in the Study Termination CRF page and any ongoing AEs at the time of study withdrawal must be followed until resolution/stabilization.

If the clinical study is prematurely terminated by the Sponsor, the investigator is to promptly inform the study subjects and local EC/IRB and should assure appropriate therapy and follow up for the subjects. All procedures and requirements pertaining to the archiving of study documents should be followed. All other study materials (study medication/vaccines, etc.) must be returned to the Sponsor.

For subjects who are withdrawn from the study due to receipt of an excluded medication/vaccination or due to significant protocol non-compliance, this reason should be noted in the Study Termination CRF page.

In studies that involve more than 1 consecutive dose of study vaccine, a separate event is “**withdrawal of study vaccination**”. This event may occur if subjects are expected to receive more than 1 consecutive dose of vaccine as part of study participation. The act of withholding additional study vaccinations is referred to as withdrawal of study vaccination. Subjects may be withdrawn from study vaccination for several reasons including but not limited to: AE related to earlier vaccinations, failure to meet inclusion or exclusion criteria for revaccination (see [section 4.0, Selection Of Study Population](#)), or pregnancy. **Subjects who are withdrawn from study vaccination should be encouraged to continue in the study for safety follow-up and other procedures as appropriate until the scheduled termination visit.** If the subject is withdrawn from study vaccination(s) due to adverse event, this event must be linked to the withdrawal from vaccination on the AE CRF page

Protocol Deviation

A protocol deviation is any change, divergence, or departure from the study design or procedures of a study protocol. In general, subjects associated with protocol deviations may remain in the study unless continuation in the study jeopardizes the subject’s health, safety, or rights.

Investigators will apply due diligence to avoid protocol deviations. Under no circumstances should the investigator contact SBVGH or its agents, if any, monitoring the study to request approval of a protocol deviation, as no authorized deviations are permitted. If the investigator feels a change to the protocol would improve the conduct of the study this must be considered a protocol amendment, and unless such an amendment is agreed upon by SBVGH and approved by the IRB/EC and health authorities it cannot be implemented.

Any subject who becomes pregnant during the study, despite the protocol requirement for adequate contraception, will not receive further vaccination but should be encouraged to continue participating in the study for safety follow-up. The site must complete a paper Pregnancy Report CRF (initial report) as soon as possible after learning of pregnancy occurrence (see [section 7.1.6, Pregnancies](#) for further details). If the subject withdraws from the study for any of the above categories except death, the site will obtain permission from the subject to continue to remain in contact with her until the outcome of the pregnancy is known, even if the outcome is not known until after the subject reaches the end of follow-up period.

Refer to [section 3.6, Stopping/Pausing Guidelines](#) and to [section 3.7 Procedures related to neutropenia](#) for further details in case of neutropenia.

3.10 End of Study

Most clinical trials intended to support the efficacy/immunogenicity and safety of an Investigational Product proceed to full completion of planned sample size accrual.

Evaluation of the primary and/or secondary immunogenicity/efficacy objectives requires the testing of biological samples from the study subjects, which can only be completed after all samples are collected. The last samples for the analysis of the primary and/or secondary objectives will be taken at visit 5. For the purpose of this protocol, end of study is defined as the completion of the testing of such biological samples, to be achieved no later than 8 months after collection of the last biological sample visit 5.

4. SELECTION OF STUDY POPULATION

4.1 Inclusion Criteria

1. Individuals ≥ 18 years to ≤ 45 years of age on the day of informed consent who are resident in the study area and are not planning to leave during the study period.
2. Individuals who, after the nature of the study has been explained, have voluntarily given written consent according to local regulatory requirements, prior to study entry.
3. Individuals who can comply with study procedures including follow-up¹.
4. Individuals in good health as determined by the outcome of medical history, physical examination, hematology, renal function, and liver function tests, urine dipstick/urinalysis and the clinical judgment of the investigator.

5. Males

Or

Females of childbearing potential who are using an effective birth control method² which they intend to use for the duration of the study

Or

Females without childbearing potential (i.e. irrespective of birth control method)

Prior to receipt of second study vaccination, subjects must be evaluated to confirm that they are eligible for subsequent vaccination. If subjects do not meet any of the original inclusion criteria listed above, they should not receive additional vaccinations.

4.2 Exclusion Criteria

1. Individuals with behavioral or cognitive impairment or psychiatric disease that, in the opinion of the investigator, may interfere with the subject's ability to participate in the study.
2. Individuals with any progressive or severe neurological disorder, seizure disorder or previous Guillain-Barré syndrome.

¹ A subject is considered to be compliant if the Investigator judges that the subject will return for all the follow-up visits as scheduled in the study.

² The following birth control methods are considered effective:

- Hormonal contraceptive (such as oral, injected or implantable contraceptives) if used for at least 2 months prior to 1st vaccination

3. Individuals who, in the judgment of the investigator, may not be able to comply with all the required study procedures.
4. Individuals with history of any illness that, in the opinion of the investigator, might interfere with the results of the study or pose additional risk to the subjects due to participation in the study.
5. Individuals with history of reactive arthritis.
6. Individuals with known HIV or hepatitis B virus infection or HIV related disease, history of an autoimmune disorder or any other known or suspected impairment /alteration of the immune system. Individuals under systemic administration of corticosteroids (PO/IV/IM) for more than 14 consecutive days within 90 days prior to screening.
7. Individuals with a known bleeding diathesis, or any condition that may be associated with a prolonged bleeding time.
8. Individuals with a neutrophil count lower than $1.8 \times 10^9/L$ (applicable to the initial 18 subjects) or lower than $1.0 \times 10^9/L$ (applicable to the additional subjects if approved by DSMB) at screening
9. Individuals with any serious chronic or progressive disease according to judgment of the investigator (e.g., neoplasm, insulin dependent diabetes, Type 2 diabetes mellitus, hypertension, cardiac, renal or hepatic disease and tuberculosis).
10. Individuals who have any malignancy or lymphoproliferative disorder.
11. Individuals with history of allergy to vaccines components or any other allergies deemed by the investigator to increase the risk of an adverse event if they were to participate in the trial.
12. Individuals participating in any clinical trial with another investigational product within 28 days prior to the screening study visit or intent to participate in another clinical study at any time during the conduct of this study.
13. Individuals who received vaccines containing meningococcal A, C, W, Y or tetanus, diphtheria or pertussis antigens within 12 months before screening, or any other vaccines within 4 weeks prior to screening in this study or who are planning to receive any vaccine within the entire study duration.
14. Individuals who have received blood, blood products, and/or plasma derivatives including parenteral immunoglobulin preparations in the 12 weeks prior to the first dose of the study vaccine.
15. Individuals who are study personnel or immediate family members (parents, children, spouse and brothers/sisters) to the personnel conducting this study.

16. Individuals with body temperature $> 38.0^{\circ}\text{C}$ within 3 days of intended study vaccination is a reason for delay of vaccination (see [section 4.3 Criteria for Delay of Vaccination](#) for further details).
17. Individuals with Body Mass Index (BMI) $> 30 \text{ kg/m}^2$
18. Individuals with history of substance or alcohol abuse within the past 2 years.
19. Women who are pregnant or are breast-feeding, or are of childbearing age who have not used (for the two months preceding the 1st vaccination) and are not willing to use acceptable contraceptive measures, for the duration of the study. If subjects are women of childbearing potential, they must have a negative pregnancy test at screening visit and prior to enrollment (visit 1). For the purposes of this study acceptable methods of contraception are oral, injected or implantable contraceptives.
20. Individuals who have a previously laboratory confirmed case of disease caused by *S. sonnei*.
21. Any condition which, in the opinion of the investigator, may pose an increased and unreasonable safety risk to the subject if they participated in the study.

Prior to receipt of second study vaccination, subjects must be evaluated to confirm that they are eligible for subsequent vaccination. If subjects meet any of the original exclusion criteria listed above, they should not receive additional vaccinations.

4.3 Criteria for Delay of Vaccination

There may be instances when individuals meet all eligibility criteria for vaccination yet have a transient clinical circumstance which may warrant delay of vaccination:

- body temperature elevation [$\geq 38.0^{\circ}\text{C}$ ($\geq 100.4^{\circ}\text{F}$) within 3 days prior to intended study vaccination]
- use of antipyretics and/or analgesic medications within 24 hours prior to vaccination.
- Individuals who have received blood, blood products and/or plasma derivatives or any parenteral immunoglobulin preparation in the past 12 weeks.

Under such circumstances, a subject may be considered eligible for study enrolment after the appropriate window for delay has passed and inclusion/exclusion criteria have been rechecked, and if the subject is confirmed to be eligible.

5. STUDY PROCEDURES

The sections that follow provide an overview of the procedures that are to be followed in enrolling, evaluating, and following subjects who participate in this clinical study. Visits can be either clinic visits or home visits, as specified in the Table below and in the [Time and Events Table 2](#).

Table 5-1 Study Procedures

Visit Category	Procedures
Pre-vaccination Clinic Visit(s)	Section 5.1 describes procedures to be followed prior to study vaccination: informed consent, screening, enrolment, and randomization
Vaccination Clinic Visit(s)	Section 5.2 describes procedures to be followed during each clinic visit involving vaccination: vaccination, post-vaccination procedures, and post-vaccination reminders
Post-vaccination Visit(s)	Section 5.3 describes follow-up clinic and home visits
Unscheduled Visit(s)	Section 5.4 describes possible procedures to be followed at unscheduled clinic visit
Study Termination Visit	Section 5.5 describes procedures to be followed at the last study visit for a subject (may include early termination visit)

5.1 Pre-vaccination Clinic Visit(s)

This section describes the procedures that must be performed for each potential subject prior to vaccination, including obtaining informed consent, screening, enrolment and randomization.

5.1.1 Informed Consent/Assent

"Informed consent" is the voluntary agreement of an individual or his/her legal guardian(s) to participate in research. Consent must be given with free will of choice, and without undue inducement. The individual must have sufficient knowledge and understanding of the nature of the proposed research, the anticipated risks and potential benefits, and the requirements of the research to be able to make an informed decision.

Informed consent following local IRB/EC guidance **must** be obtained before conducting any study-specific procedure (i.e., all of the procedures described in the protocol). The process of obtaining informed consent should be documented in the subject source document in addition to maintaining a copy of the signed and dated informed consent.

Study will be explained in either Swahili or in English, where prospective participants express a preference. If a subject is unable to read, an impartial witness should be present during the entire informed consent discussion. An impartial witness is defined as a person who is independent from study conduct, who cannot be unfairly influenced by those involved with the study, who attends the informed consent process if the subject or the subject's legally acceptable representative cannot read, and who reads the informed consent form and any other written information supplied to the subject. After the written informed consent form and any other written information to be provided to subjects, is read and explained to the subject and after the subject has verbally consented to the subject's participation in the study and, if capable of doing so, has signed and personally dated the informed consent form, the witness should sign and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the subject and that informed consent was freely given by the subject.

Pre-test counselling will be performed for individuals who consent to HIV testing. Those individuals, who will be found to be HIV positive, will be also counselled post-test and referred to the Ministry of Health HIV care services.

5.1.2 Screening

After an individual has consented to participate in the study and informed consent is signed, that individual will be given a unique 5-digit Screening Number (assigned sequentially by the site from ^{PPD}). The subject's unique Screening Number will be documented in the Screening and Enrolment log. The eligibility of the subject will be determined based on the inclusion and exclusion criteria listed in [section 4, Selection of Study Population](#) and evaluated during this screening procedure.

Prior to study enrolment, demographic data will be collected from the subject, including gender, race, body mass index.

Medical history will also be collected, including but not limited to any medical history that may be relevant to subject eligibility for study participation such as prior vaccinations, concomitant medications, and previous and ongoing illnesses or injuries. Relevant medical history can also include any medical history that contributes to the understanding of an adverse event that occurs during study participation, if it represents an exacerbation of an underlying disease/pre-existing problem.

Review of systems is a structured interview that queries the subject as to any complaints the subject has experienced across each organ system. This will be performed before enrolment and used to guide physical examination.

If applicable, prior and concomitant medications or vaccinations taken prior to start of study should be collected (refer to [section 6.5, Prior and Concomitant Medications and Vaccines](#) for further details).

Collect vital signs, heart rate, respiratory rate, blood pressure, and temperature. Measure height and weight.

Perform pregnancy testing in women of childbearing age (refer to [section 3.5, Collection of Clinical Specimens](#) for guidance regarding the procedure). Informed consent process with any women of childbearing potential will include counseling about pregnancy and family planning, including discussion of their commitment to practice acceptable birth control measures (defined as oral, injected or implantable contraceptives). Women of childbearing potential are defined as a post onset of menarche and pre-menopausal female capable of becoming pregnant. This does not include females who meet any of the following conditions: (1) menopause at least 2 years earlier, (2) tubal ligation at least 1 year earlier, (3) total hysterectomy or (4) post bilateral oophorectomy.

A general physical examination is to be performed by a qualified health care practitioner. “Qualified health care practitioner” refers to any licensed health care professional who is permitted by national policy to perform physical examinations and who is identified within the Study Staff Signature Log.

These data will be written in the source document (see [section 9.1, Source Documentation](#)).

Approximately 10 mL of blood will be drawn from all subjects for the hematology, renal and liver function testing, serological tests for HBV and HIV and pregnancy test (Refer to [section 3.5, Collection of Clinical Specimens](#) and to [synopsis Table 3, Hematological, Haematochemical Blood Tests and Urinalysis Table](#)).

In the event that the individual is determined ineligible for study participation, he/she is considered a screen failure. The reason for screen failure must be documented in the Screening and Enrolment log. If the individual is determined to be eligible for the study, he/she will be enrolled into the study.

5.1.3 Enrolment

If an individual is determined to be eligible for study participation, the investigator will enroll the subject

5.1.4 Randomization

Enrolled subjects will be randomized and automatically assigned a unique Subject ID. The Subject ID will be the subject's unique identification number for all CRFs and associated study documentation that will be used for duration of the study. After randomization, the Screening Number ceases to be used and remains in the Screening and Enrolment Log only.

At randomization, the subject will be assigned also a unique subject code. The subject code consists of the 2 letters subject's initials (the 1st letter of the surname name – followed by the 1st letter of the 1st name).

If for any reason, after signing the informed consent form (ICF), the subject who is eligible and enrolled fails to be randomized, this is called a randomization failure and the early termination study procedures must be applied. The reason for all randomization failures should be recorded in the Screening and Enrolment Log and in the source document as specified in the Source Data Agreement (SDA). The information on subjects who are randomization failures should be kept distinct from subjects who are screen failures, as described in [section 5.1.2, Screening](#).

If for any reason, after randomization the subject fails to undergo treatment, this is an Early Termination and the reason should be recorded in source document as specified in the SDA. The information on these Early Termination subjects should be kept distinct in the source documentation from randomization failures.

Screening phase will be performed until 72 subjects are randomized and vaccinated. Additional subjects may be randomized into the study at the discretion of the sponsor in the case of any subject who is randomized but does not receive any study vaccine. Subjects withdrawn or lost to follow up will not be replaced.

5.2 Vaccination Clinic Visit(s)

Vaccination will be performed on day 1 and day 29.

For studies which have visits for concomitant vaccinations or treatments, see [section 6.5, Prior and Concomitant Medications and Vaccines](#) for those visit procedures.

Ensure all serology samples are taken **prior** to each vaccination.

After completing the pre-vaccination procedures on day 1, administer the vaccine to the subject according to the procedures described in [section 6.3, Vaccine Preparation and Administration](#). Observe the blinding procedures described in [section 3.3, Blinding Procedures](#).

Prior to administration of each vaccination, confirm that the subject is eligible for additional study vaccinations and does not meet any criteria for delaying additional study vaccinations as described in [section 4, Selection of Study Population](#).

5.2.1 Post-vaccination Procedures

The following post-vaccination procedures will be performed on day 1 and day 29.

After vaccination, the subject will be observed for at least 1 hour including observation for unsolicited adverse events, solicited adverse events, and body temperature measurement. Record all safety data collected during this time in the subject's source document.

The site should schedule the next study activity (daily home visits) with the subject.

The subject should be reminded of the next planned study activity. The subject will be reminded to contact the site if there are any questions, and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical condition that leads to a hospitalization or an emergency room visit or to a visit to/by a doctor or is of concern.

5.2.2 Post-vaccination Reminders

Not Applicable

5.3 Post-vaccination Visit(s)

5.3.1 Follow-up Home Visit(s)

Post-vaccination home visits will be performed daily by a field worker from day 1 (6 hours after vaccination) to day 7 to check solicited local and systemic adverse events and medications/vaccinations received, following discussion with the subject.

A Diary Card collected in person by study personnel will be used in this study to document solicited adverse events. The Diary Card is the only source for collection of these data; therefore, it is critical that the trained site staff completes the subject's Diary Card correctly. The appropriate site staff should be trained on how and when to complete each field of subject's Diary Card.

The appropriate site staff should be trained on how to measure local solicited adverse events and body temperature. The trained field workers who will perform the home visits will be instructed to direct the subject to the clinic if the event requires medical

supervision. The measurement of solicited local adverse events is to be performed using the ruler provided by the site.

The appropriate site staff should be instructed how to perform body temperature measurement using the thermometer provided by the site. The temperature recorded in the Diary Card will be the one measured by the site staff during point of contact with subject at home.

Appropriate training should be directed at the site staff who will perform the measurements of adverse events and who will enter the information into the subject's Diary Card. The identity of the person who will enter the information into the Diary Card must be documented in the subject's source record and in the study delegation log. Any individual that makes entries into the Diary Card must receive training on completion of the Diary Card. This training must be documented.

Whenever possible, the same individual should complete the subject's Diary Card throughout the course of the study.

5.3.2 Follow-up Clinic Visit(s)

Safety follow-up clinic visits will be performed 7 days after V1 and V3.

During the follow-up clinic visit, the subject's source document and Diary Card will be reviewed. No changes to the information recorded by the trained study staff within the section of the Diary Card are permissible. For details see [sections 3.4.2, Tools Used for Data Collection](#) and [5.2.1, Post-vaccination Procedures](#). The subject will be interviewed to determine if any unsolicited adverse events occurred and if any concomitant medications or vaccines were taken/received in the time since the last clinic visit. This interview will follow a script which will facilitate the collection of relevant safety information. The healthcare professional reviewing these data will discuss the symptoms (if any) reported by the subject and will determine if any additional diagnoses and/or adverse events are present. Adverse events reported by the subject at this follow-up clinic visit must be recorded in the subject's source document and on an Adverse Events CRF, as specified in [section 7.1, Safety Assessment](#), and not written on the script used for the interview.

Perform a brief symptom-directed physical examination if necessary according to symptoms the subject has reported. This is a physical examination that will include an examination of organ systems that are relevant to the investigator based on review of the subject's reported adverse events, concomitant medication use. This assessment may include: measurement of vital signs, body temperature taken axillary and a check of general appearance. The physical assessment must be performed by the investigator or

designee of the investigator, who is qualified to perform a physical assessment in accordance with their institutional policy. Corresponding information is documented in the subject's source document and CRF(s).

At this visit blood sample for safety evaluation and urine sample for urine dipstick/urinalysis will be obtained as described in [section 3.5, Collection of Clinical Specimens](#)

The site should schedule the next study clinic visit with the subject. The subject will receive a reminder of the next planned study activity. The subject will be reminded to contact the site if there are any questions and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical condition that leads to a hospitalization or an emergency room visit.

5.3.3 Safety Follow-up Calls

Not Applicable

5.4 Unscheduled Visits

An unscheduled visit describes a non-routine study visit triggered by a specific event. These could include anticipated or unanticipated adverse events or interventions.

All individuals with a neutropenia, occurring at any time during the study, will have additional blood draws for complete blood count to be repeated on a weekly basis until the neutropenia resolves. If neutropenia occurs at the subject's last study visit, the complete blood count will be repeated on a regular basis until resolution. For classification of neutropenia during the trial, refer to [Section 3.7, Procedures Related To Neutropenia](#).

5.5 Study Termination Visit

The study termination visit 5 will occur 28 days after 2nd vaccination. The termination visit may be a clinic visit or a home visit. The date of termination is the date of the last contact (clinic visit or home visit) in which the subject's health status was assessed or, in cases where the subject does not agree to any further safety follow-up, it is the date consent is withdrawn. This date should be recorded on the termination CRF page. For visit procedures to be performed for a subject whose planned study participation ends prematurely, please see [section 5.5.1, Early Termination Visit](#).

At the clinic visit, the following procedures will be performed:

- review of subject's medical records and source document designed to collect solicited AE,
- review of systems, interview of subject to collect unsolicited adverse events, medically attended adverse events, AEs leading to withdrawal, SAEs, AESIs, and new onset of chronic disease,
- interview of subject to collect concomitant medications/vaccinations,
- symptom-directed physical assessment including measurement of vital signs and a check of general appearance,
- Blood sampling for immunogenicity and safety laboratory assessment,
- Urine sample for urine dipstick/urinalysis and pregnancy testing.

All individuals with neutropenia either ongoing or with onset at visit 5 (last study visit) will have additional blood draws for complete blood count to be repeated on a regular basis until the neutropenia resolves (for classification of neutropenia during the trial, refer to [Section 3.7, Procedures Related To Neutropenia](#)). Laboratory results obtained after study termination will be maintained in subject medical records and not entered in the CRF.

When the clinical study report is completed, the investigators will share the summary results with the participating communities. Town hall and village meetings will be held to convey the information and to allow for questions and answers.

The site will complete the termination CRF page and this will mark the completion of the subject's participation in the study.

5.5.1 Early Termination Visit

When a subject is withdrawn from treatment or withdraws from the study, the investigator will notify the Sponsor and, when possible, will perform the procedures listed below. The reason(s) for the early termination will be included in the subject's source documentation. Early Termination Visits include subjects who were randomized but not treated.

At the clinic visit or during the home visit, the following procedures will be performed (if the Early Termination Visit is a home visit, collect as much information as possible):

- review of subject's medical records, source document and Diary Card,

- review of systems, interview of subject to collect unsolicited adverse events, medically attended adverse events, AEs leading to withdrawal, SAEs, AESIs, and new onset of chronic disease,
- interview of subject to collect concomitant medications/vaccinations,
- symptom-directed physical assessment including measurement of vital signs and a check of general appearance,
- blood sampling for safety laboratory assessment (no blood for immunogenicity assessment should be obtained unless this is agreed in advance with the sponsor),
- urine sample for urine dipstick/urinalysis and pregnancy testing.

All individuals with neutropenia either ongoing or with onset at last study visit will have additional blood draws for complete blood count to be repeated on a regular basis until the neutropenia resolves (for classification of neutropenia during the trial, refer to [Section 3.7, Procedures Related To Neutropenia](#)). Laboratory results obtained after study termination will be maintained in subject medical records and not entered in the eCRF.

The site will complete the termination CRF page and this will mark the completion of the subject's participation in the study.

6. TREATMENT OF SUBJECTS

All vaccines associated with this study are to be stored separately from other vaccines and medications in a secure location under appropriate storage conditions with temperature monitoring. **All vaccines associated with this study must be checked for expiration date prior to use. Expired vaccines must not be administered to subjects.**

6.1 Study Vaccine(s)

The term 'study vaccine' refers to those vaccines provided by the Sponsor, which will be evaluated as part of the study objectives. The study vaccines specific to this study are described below.

SBVGH *S. sonnei* (1790GAHB) vaccine

The investigational agent is the SBVGH *S. sonnei* vaccine. The vaccine consists of *S. sonnei* 1790-GMMA (approximately 200 µg/mL, measured by protein content) adsorbed to Alhydrogel (0.7 mg Al³⁺/mL) in Tris-buffered saline. The vaccine does not contain any preservative and is available as a liquid formulation in single dose vials with 0.7 mL of injectable solution containing approximately 140 µg of GMMA (as protein content), adsorbed onto 0.49 mg Al³⁺.

The vaccine will be used at two different antigen doses obtained by bed-side mixing. Following dilution with Alhydrogel (0.7 mg Al³⁺/mL) in Tris-buffered saline, the volume administered will be 0.5 mL for both doses:

Group A: Each 0.5 mL dose of 1790GAHB will contain approximately 25 µg of GMMA total protein and 0.35 mg of Al³⁺.

Group B: Each 0.5 mL dose of 1790GAHB will contain approximately 100 µg of GMMA total protein and 0.35 mg of Al³⁺.

In each group two vaccinations, 28 days apart, will be administered intramuscularly.

Bed-side mixing instructions will be provided to the investigator and will be located in the investigator site file.

Control vaccines

Menveo: vaccine indicated for active immunization to prevent invasive meningococcal disease caused by *Neisseria meningitidis* serogroups A, C, Y and W-135. The vaccine contains no preservative or adjuvant.

Each 0.5 mL dose of vaccine contains 10 µg MenA oligosaccharide, 5 µg of each of MenC, MenY and MenW-135 oligosaccharides conjugated to 32.7 to 64.1 µg CRM₁₉₇ protein.

Boostrix: Vaccine indicated for active booster immunization against tetanus, diphtheria, and pertussis. It contains tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis antigens (inactivated pertussis toxin [PT], formaldehyde-treated filamentous hemagglutinin [FHA] and pertactin). Each antigen is individually adsorbed onto aluminum hydroxide.

For both control vaccines, the Summary of Product Characteristics (SPC) will be provided to the investigator and will be located in the investigator site file.

6.2 Non-Study Vaccines

The term ‘non-study vaccine’ refers to those vaccines which will be intentionally given to study subjects but not formally included in the analysis of study objectives.

This protocol does not foresee the use of non-study vaccines.

6.3 Vaccine Preparation and Administration

The investigator or designee will be responsible for oversight of the administration of vaccine to subjects enrolled in the study according to the procedures stipulated in this study protocol. All vaccines will be administered only by unblinded personnel who are qualified to perform that function under applicable local laws and regulations for the specific study site.

PRECAUTIONS TO BE OBSERVED IN ADMINISTERING STUDY VACCINE:

Prior to vaccination, subjects must be determined to be eligible for study vaccination and it must be clinically appropriate in the judgment of the investigator to vaccinate. Eligibility for vaccination prior to first study vaccine administration is determined by evaluating the entry criteria outlined in protocol [sections 4.1, Inclusion Criteria](#) and [4.2, Exclusion Criteria](#).

Eligibility for subsequent study vaccination is determined by following the criteria outlined in [section 4.3, Criteria for Delay of Vaccination](#).

Study vaccines should not be administered to individuals with known hypersensitivity to any component of the vaccines. Standard immunization practices are to be observed and care should be taken to administer the injection intramuscularly. Before administering vaccine, the vaccination site is to be disinfected with a skin disinfectant (e.g., 70% alcohol). Allow the skin to dry. **DO NOT inject intravascularly.**

As with all injectable vaccines, trained medical personnel and appropriate medical treatment should be readily available in case of anaphylactic reactions following vaccine administration. For example, epinephrine 1:1000, diphenhydramine, and/or other medications for treating anaphylaxis should be available.

6.4 Vaccine Administration Error or Overdose of Vaccine

Vaccine administration error is defined as receiving a dose of study vaccine that was not reconstituted as instructed or administered by a different route from the intended route of administration. An overdose of study vaccine (whether accidental or intentional) is defined when a dose higher than the recommended dose is administered in one dose of study vaccine.

An overdose would also occur if two doses of the study vaccine are administered within half the time of the recommended interval between doses, as defined in the protocol i.e. for this protocol, both an overdose and vaccine administration error would occur if receipt of more than one dose takes place in a 12 day period.

Any vaccine administration error or overdose of study vaccine detailed in this protocol must be reported as an adverse event, and if the vaccine administration error or overdose is associated with a serious adverse event, it must be reported as such within 24 hours to the Sponsor.

6.5 Prior and Concomitant Medications and Vaccines

All medications, vaccines and blood products taken or received by the subject within 4 weeks prior to the start of the study are to be recorded on the Prior and Concomitant Medications CRF.

The use of antipyretics and/or analgesic medications within 24 hours prior to vaccination must be identified and the reason for their use (prophylaxis versus treatment) must be described in the source document and Concomitant Medications CRF. Use of antipyretics/analgesics within 24 hours prior to vaccine administration is a reason to delay study vaccination (see [section 4.3, Criteria for Delay of Vaccination](#)).

Medications taken for prophylaxis are those intended to prevent the onset of symptoms. Medications taken for treatment are intended to reduce or eliminate the presence of symptoms that are present.

Concomitant medications include all medications (including vaccines) taken by/administered to the subject at and after enrolment and must be documented on the Concomitant Medications CRF.

When recording concomitant medications/vaccines, they should be checked against the study entry and continuation criteria in [section 4, Selection of Study Population](#) to ensure that the subject should be enrolled/continue in the study.

6.6 Vaccine Supply, Labeling, Storage and Tracking

The Sponsor will ensure the following:

- Supply the study vaccine(s).
- Appropriate labeling of all study vaccines provided that complies with the legal requirements of each country where the study is to be performed.

The investigator must ensure the following:

- Acknowledge receipt of the study vaccines by a designated staff member at the site, including:
 - Confirmation that the vaccines were received in good condition
 - Confirmation to the Sponsor of the temperature range during shipment from the Sponsor to the investigator's designated storage location
 - Confirmation by the Sponsor that the vaccines are authorized for use.
- Proper storage of the study vaccines, including:
 - Storage in a secure, locked, temperature-controlled location.
 - Proper storage according to the instructions specified on the labels.
 - Appropriate record keeping and inventory of the study vaccines, including regular documentation of adequate storage temperature.
- Appropriate use of the study vaccines, including:
 - Not use of vaccines prior to receipt of authorization for use from the Sponsor.
 - Use only in accordance with the approved protocol.
 - Dilution in accordance to bed-side mixing procedure and documentation
 - Proper handling, including confirmation that the vaccine has not expired prior to administration.
 - Appropriate documentation of administration of vaccines to study subjects including:
 - Date, dosage, batch/lot numbers, expiration dates, unique identifying numbers assigned to subjects and study vaccines, and time of vaccine administration.

This information will be maintained in an accountability log that will be reviewed by the site monitor.

- Reconciliation of all vaccines received from the Sponsor. Reconciliation is defined as maintaining records of which and how many vaccines were received, which vaccines (and dilution for *S. sonnei* vaccine) were administered to subjects, which vaccines were destroyed at the site, and which vaccines were returned to the Sponsor, as applicable.
- Proper adherence to the local institutional policy with respect to destruction of study vaccines.
- Complete record keeping of vaccine use, wastage, return or destruction, including documentation of:
 - Copy of the site's procedure for destruction of hazardous material.
 - Number of doses destroyed, date of destruction, destruction code (if available), method of destruction, and name of individual performing destruction.

Vaccines that have been stored differently from the manufacturer's indications **must not** be used unless the Sponsor provides written authorization for use. In the event that the use cannot be authorized, the Sponsor will make every effort to replace the vaccine supply. All vaccines used in conjunction with this protocol must be stored separately from normal hospital/practice stocks to prevent unintentional use of study vaccines outside of the clinical study setting.

Monitoring of vaccine accountability will be performed by the study monitor during site visits and at the completion of the study.

At the conclusion of the study, and as appropriate during the course of the study, the investigator must ensure that all unused study vaccines, packaging and supplementary labels are destroyed locally (upon approval from Sponsor) or returned to the Sponsor.

7. ASSESSMENTS

7.1 Safety Assessment

The measures of safety used in this study are based on previous study data. They include a close vigilance for, and stringent reporting of selected local and systemic adverse events routinely monitored in vaccine studies as indicators of reactogenicity.

An adverse event (AE) is defined as any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product at any dose that does not necessarily have to have a causal relationship with this treatment. Therefore, an AE can be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the investigational product. This definition includes intercurrent illnesses or injuries and exacerbation of pre-existing conditions.

The period of observation for AEs extends from the time the subject signs informed consent until he or she completes the specified safety follow-up period (28 days after each vaccination) or terminates the study early (whichever comes first). AEs occurring after the informed consent form is signed but prior to receiving study vaccine/product will be documented as an adverse event and recorded within source document. However, any AEs occurring prior to receipt of any study vaccine will be analyzed separately from “treatment emergent” AEs (AEs occurring after administration of the first study vaccine).

Adverse events are collected as either solicited or unsolicited adverse events. Solicited events are derived from organized data collection systems, such as Subject interview.

7.1.1 Solicited Adverse Events

The term “reactogenicity” refers to solicited signs and symptoms (“solicited adverse events”) occurring in the hours and days following a vaccination, to be collected by the subject for 7 consecutive days.

The following solicited adverse events are included in the Diary Card that will be used in this study to document solicited adverse events.

Solicited local adverse events: erythema, induration and pain at injection site.

Solicited systemic adverse events: headache, arthralgia, chills, fatigue, malaise, myalgia, and fever (body temperature measured axillary).

Other solicited reactions: Use of analgesics/antipyretics, body temperature (measured axillary).

Each adverse event is to be assessed using the scoring system reported below:

Solicited Local Adverse Events

Solicited local adverse events	Grade 0 Absent	Present - Grading of Severity		
		Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe
Injection site Erythema (Captured as measurements in millimeters)	1-24 mm	25-50 mm	51-100 mm	> 100 mm
Injection site Induration (Captured as measurements in millimeters)	1-24 mm	25-50 mm	51-100 mm	> 100 mm
Injection site Pain	No pain	Present but does not interfere with activity	Interferes with activity	Prevents daily activity

Solicited Systemic Adverse Events

Solicited systemic adverse events	Grade 0 Absent	Present - Grading of Severity*		
		Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe
Headache	No headache	Present but does not interfere with activity	Interferes with activity	Prevents daily activity
Arthralgia	No arthralgia	Present but does not interfere with activity	Interferes with activity	Prevents daily activity
Chills	No chills	Present but does not interfere with activity	Interferes with activity	Prevents daily activity
Fatigue	No fatigue	No interference with activity	Some interference	Significant; prevents daily

			with activity	activity
Malaise	No malaise	No interference with activity	Some interference with activity	Significant; prevents daily activity
Myalgia	No myalgia	Present but does not interfere with activity	Interferes with activity	Prevents daily activity
Fever as a Body temperature (Captured as measurements in degrees Celsius)	$\leq 37.9^{\circ}\text{C}$	$\geq 38.0 - 38.9^{\circ}\text{C}$	$\geq 39.0 - 39.9^{\circ}\text{C}$	$\geq 40.0^{\circ}\text{C}$
	Axillary Temperature: $<35.5^{\circ}\text{C}$ to $\geq 38.0^{\circ}\text{C}$			

Other Solicited Adverse Events

Other Solicited adverse events	Grade 0 Absent	Present - Grading of Severity		
		Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe
Use of analgesics/antipyretics	Categorized as “yes” or “no”			

The study staff must review the data entered into the Diary Card as described in [section 3.4.2, Tools Used for Data Collection](#) and [section 5.3.1, Follow-up Clinic Visit\(s\)](#).

Note: Any solicited adverse event that meets any of the following criteria must be entered into subjects’ source document (see [section 9.1, Source Documentation](#)) and also as an adverse event on the Adverse Event CRF:

- Solicited local or systemic adverse event that continues beyond day 7 after vaccination.
- Solicited local or systemic adverse event that leads to a visit to a healthcare provider (medically attended adverse event, see [section 7.1.3, Evaluation of Adverse Events](#)).
- Solicited local or systemic adverse event leading to the subject withdrawing from the study or the subject being withdrawn from the study by the investigator (adverse event leading to withdrawal, see [section 7.1.3, Evaluation of Adverse Events](#)).
- Solicited local or systemic adverse event that otherwise meets the definition of a serious adverse event (see [section 7.1.4, Serious Adverse Events](#)).

7.1.2 Unsolicited Adverse Events

An unsolicited adverse event is an adverse event that was not solicited using the Diary Card and that was spontaneously communicated by a subject who has signed the informed consent.

Potential unsolicited AEs may be medically attended (defined as symptoms or illnesses requiring hospitalization, or emergency room visit, or visit to/by a health care provider), or were of concern to the subject. In case of such events, subjects will be instructed to contact the site as soon as possible to report the event(s). The detailed information about the reported unsolicited AEs will be collected by the qualified site personnel during the interview and will be documented in the subject's records.

Unsolicited AEs that are not medically attended nor perceived as a concern by subjects will be collected during interview with the subject and by review of available medical records at the next visit (see section 5.3, [Post-vaccination Visit\(s\)](#)).

7.1.3 Evaluation of Adverse Events

Every effort should be made by the investigator to evaluate safety information reported by a subject for an underlying diagnosis and to capture this diagnosis as the event in the AE page. In other words, the practice of reporting only symptoms (e.g., "cough" or "ear pain") are better reported according to the underlying cause (e.g., "asthma exacerbation" or "otitis media").

The severity of events reported on the Adverse Events CRF will be determined by the investigator as:

Mild:	transient with no limitation in normal daily activity.
Moderate:	some limitation in normal daily activity.
Severe:	unable to perform normal daily activity.

The relationship of the study treatment to an AE will be determined by the investigator based on the following definitions:

1. Not Related

The AE is not related to an investigational vaccine if there is evidence that clearly indicates an alternative explanation. If the subject has not received the vaccine, the timing of the exposure to the vaccine and the onset of the AE are not reasonably related in time, or other facts, evidence or arguments exist that reasonably suggest an alternative explanation, then the AE is not related.

2. Possibly Related

The administration of the investigational vaccine and AE are considered reasonably related in time and the AE could be explained by exposure to the investigational vaccine or by other causes.

3. Probably Related

Exposure to the investigational vaccine and AE are reasonably related in time and no alternative explanation has been identified.

The relationship of the study treatment to an unsolicited AE will be determined by the investigator.

Note: solicited AEs will not be evaluated for relationship to study treatment. Grading for severity of solicited local and systemic AEs is described in [section 7.1.1, Solicited Adverse Events](#).

Adverse events will also be evaluated by the investigator for the co-existence of any of the other following conditions:

- “Medically attended adverse event”: an adverse event that leads to a visit to a healthcare provider.
- “New onset of chronic disease” (NOCD): an adverse event that represents a new diagnosis of a chronic medical condition that was not present or suspected in a subject prior to study enrolment.
- AEs leading to withdrawal: adverse events leading to study or vaccine withdrawal.

If solicited or unsolicited adverse events have been reported and the subject indicated that the symptoms required medical attendance or were of concern, the subject must be contacted for further information.

When the subject is contacted for any of these reasons, the contact must be documented in the subject’s source documentation.

All AEs, regardless of severity, will be monitored until resolution or until the investigator assesses them as chronic or stable. All subjects experiencing AEs - whether considered associated with the use of the study vaccine or not - must be monitored until symptoms subside and any abnormal laboratory values have returned to baseline, or until there is a satisfactory explanation for the changes observed, or until death, in which case a full pathologist’s report should be supplied, if possible. The investigator’s assessment of

ongoing Adverse Events at the time of each subject's last visit should be documented in the subject's medical chart.

7.1.4 Serious Adverse Events

A serious adverse event (SAE) is defined as any untoward medical occurrence that at any dose results in one or more of the following:

- Death.
- Is life-threatening (i.e., the subject was, in the opinion of the investigator, at immediate risk of death from the event as it occurred); it does not refer to an event which hypothetically might have caused death if it were more severe.
- Required or prolonged hospitalization.
- Persistent or significant disability/incapacity (i.e., the event causes a substantial disruption of a person's ability to conduct normal life functions).
- Congenital anomaly/or birth defect.
- An important and significant medical event that may not be immediately life threatening or resulting in death or hospitalization but, based upon appropriate medical judgment, may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above.

Adverse events which do not fall into these categories are defined as non-serious.

It should be noted that a severe adverse event need not be serious in nature and that a serious adverse event need not, by definition, be severe.

Serious adverse events will be captured both on the Vaccines Serious Adverse Event (VSAE) form as well as on the AE CRF. All SAEs will be evaluated by the investigator for relationship of the event to study vaccine. SAEs that are judged to be possibly or probably related to the study vaccine should be reported to the Sponsor as related/suspected events.

The relationship of the study treatment to an SAE will be determined by the investigator based on the following definitions:

1. Related/suspected

The SAE is judged by the investigator to be possibly or probably related to the study vaccine on the AE CRF page (see [section 7.1.3, Evaluation of Adverse Events](#)).

2. Not Related

The SAE is not related if exposure to the study vaccine has not occurred, **or** the occurrence of the SAE is not reasonably related in time, **or** the SAE is considered unlikely to be related to use of the study vaccine, i.e., there are no facts (evidence) or arguments to suggest a causal relationship.

The relationship of the study vaccine to an SAE will be determined by the investigator.

In addition, SAEs will be evaluated by the Sponsor or designee for “expectedness.” An unexpected AE is one that is not listed in the current Summary of Product Characteristics or the Investigator’s Brochure or an event that is by nature more specific or more severe than a listed event.

In addition, a pre-existing event or condition that results in hospitalization should be recorded on the Medical History CRF. If the onset of an event occurred before the subject entered the study (e.g., any pre-planned hospitalization for conditions like cosmetic treatments or for non-emergency routine visits for a pre-existing condition), the hospitalization would not lead to an AE being classified as serious unless, in the view of the investigator, hospitalization was prolonged as a result of participation in the clinical study or was necessary due to a worsening of the pre-existing condition.

7.1.4.1 Adverse Events of Special Interest

Adverse events of special interest (AESIs) are predefined adverse events that will be specifically highlighted to the investigator and will be summarized separately at the end of the study. All AESIs occurring during the study will be categorized and reported as a SAE.

Reactive arthritis (ReA) will be collected and analyzed as an AESI for this study.

Reactive arthritis is defined as non-purulent joint inflammation that develops in response to an infection in another part of the body. Since the inflammation is triggered by a previous condition, it is termed “reactive”. Intestinal pathogens that have been associated with reactive arthritis include *Campylobacter*, *Salmonella*, *Yersinia*, *Clostridium difficile*, and *Shigella*. If reactive arthritis is caused by an auto immune response, there is at least a possibility that it could be initiated by vaccination of susceptible people with the 1790GAHB vaccine.

For diagnosis of ReA, imaging and aspiration are not required (unless clinically indicated).

7.1.5 Methods for Recording Adverse Events and Serious Adverse Events

Findings regarding Adverse Events must be reported on an Adverse Events CRF, as specified in [section 7.1.1, Solicited Adverse Events](#), and on the VSAE form, if applicable, which is part of the Investigator Site File. All findings in subjects experiencing AEs must be reported also in the subject's source document.

All SAEs which occur during the course of the study, whether considered to be associated with the study vaccination or not, must be reported **within 24 hours of the site becoming aware of the event** to SBVGH or its designee. Specific instructions and contact details for collecting and reporting SAEs to SBVGH will be provided to the investigator.

All SAEs are also to be documented on the Adverse Events CRF. Any medication or other therapeutic measures used to treat the AE will be recorded on the appropriate CRF(s) in addition to the outcome of the AE.

After receipt of the initial report, representatives of SBVGH or its designee will contact the investigator if it is necessary to obtain further information for assessment of the event.

All SAEs must be reported by the investigator to his/her IRB or applicable regulatory authorities in accordance with institutional policy/regulatory requirements and adequate documentation of this notification must be provided to the Sponsor.

SBVGH or its designee must also comply with the applicable regulatory requirement(s) related to the reporting of suspected unexpected serious adverse vaccine reactions (also known as SUSARs) to the regulatory authority(ies) and the IRB/EC. If a SUSAR or other safety signal relating to use of one of the study vaccines is reported to SBVGH or its designee, the Sponsor will communicate the information to the investigator and the investigator will be responsible for submitting this information to the IRB and other relevant authorities.

7.1.5.1 Post-Study Events

Any SAE that occurs outside of the protocol-specified follow-up period and considered to be caused by the study vaccine must be reported to SBVGH or its designee. These SAEs will be processed by SBVGH or its designee as during the course of the study, until 6 months after last subject last visit. Instructions and contact details for collecting and reporting these suspected SAEs will be provided to the investigator.

7.1.6 Pregnancies

To ensure subjects' safety, each pregnancy in a subject after study vaccination must be reported to SBVGH or delegate within 72 hours of the site learning of its occurrence. If

the subject agrees to submit this information, the pregnancy must be followed to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. This follow-up should occur even if intended duration of safety follow-up for the study has ended.

Pregnancy data must be recorded on a Pregnancy Report CRF (initial report) and Pregnancy Follow-Up CRF (outcome report) and reported to SBVGH or delegate. Instructions and contact details for submitting the Pregnancy CRFs will be provided to the investigator.

Any pregnancy outcome meeting the definition of a SAE (see [section 7.1.4, Serious Adverse Events](#)) must also be reported on the VSAE Report Form.

7.1.7 Safety Laboratory Measurements

For list of safety laboratory measurement, refer to synopsis [Table 3, Hematological, Haematochemical Blood Tests and Urinalysis Table](#).

Safety laboratory measurement will be performed as described in [Section 3.5, Collection of Clinical Specimens](#).

Significant alterations in hematology, blood chemistry and urinalysis will be clinically assessed by the investigator's medical judgment based on interpretation of deviations from institution's normal values.

Any abnormality in laboratory measurements classified as clinically significant must be reported in the Adverse Event CRF form.

If a subject is to have blood drawn and/or urine testing for safety laboratory for repeat safety assessment (e.g., in case of markedly abnormal safety laboratory parameter), investigator's medical judgment will be applied concerning values that would trigger reanalysis and frequency of repeats.

Safety laboratory assessments will be performed at the site laboratory, and results of these tests will be recorded in the source documents and in the CRF.

7.2 Efficacy Assessment

This study has no efficacy measurements.

7.3 Immunogenicity Assessment

The measure of immunogenicity used in this study i.e. IgG enzyme-linked immunosorbent assay (ELISA) in sera is standard, widely used and generally recognized as reliable, accurate, and relevant (able to describe the quality and extent of the immune response). The ELISA methodology used in this study has been adopted based on scientific consensus and has been deemed appropriate to describe the immune response against *Shigella sonnei* GMMA in this study.

Testing will be conducted by a SBVGH or designated laboratory in a blinded manner towards the treatment arm and the visit.

The measure of immunogenicity used in this study is IgG ELISA against *Shigella sonnei* OAg. The serologic assays will be conducted on serum samples and will be performed by a SBVGH or designated laboratory in a blinded manner towards the treatment arm and the visit.

For reference of visits the measurements are taken, refer to [section 3.5](#) and to the Clinical Specimen Laboratory Manual.

8. STATISTICAL CONSIDERATIONS

8.1 Endpoints

8.1.1 Primary Endpoint(s)

8.1.1.1 Primary Safety Endpoint(s)

The safety profile of the two vaccine doses and control(s) will be assessed through measurement of the following:

- a. Number and percentage of subjects with solicited local and systemic adverse reactions during 7 days following each vaccination.
- b. Numbers and percentage of subjects with deviations from normal ranges of safety laboratory data after each vaccination.
- c. Number and percentage of subjects with reported unsolicited adverse events during 28 days following each vaccination.
- d. Number and percentage of subjects with reported SAEs throughout the study duration.
- e. Number and percentage of subjects with reported reactive arthritis (AESIs).

8.1.1.2 Primary Efficacy Endpoint(s)

The study does not have primary efficacy endpoint(s).

8.1.1.3 Primary Immunogenicity Endpoint(s)

The study does not have primary immunogenicity endpoint(s).

8.1.2 Secondary Endpoint(s)

8.1.2.1 Secondary Safety Endpoint(s)

The study does not have secondary safety endpoint(s).

8.1.2.2 Secondary Efficacy Endpoint(s)

The study does not have secondary efficacy endpoint(s),

8.1.2.3 Secondary Immunogenicity Endpoint(s)

The measures of the immunogenicity outcome, (i.e., the anti-LPS *S. sonnei* serum IgG), will include:

- a. IgG Geometric mean concentrations (GMCs) pre-vaccination (Day 1), 28 days after 1st vaccination and 28 days after 2nd vaccination, as determined by Enzyme-linked Immunosorbent Assay (ELISA), and applicable geometric mean ratios between post- and pre-vaccination samples.
- b. Number and percentage of subjects with seroresponse for anti- LPS *S. sonnei* at 28 days after 1st vaccination and 28 days after 2nd vaccination

Seroresponse is aimed to define a significant increase in post vaccination samples based on the biological performance of this specific serology assay and it is defined as:

- If the baseline value is greater than 50 ELISA Units (EU) then an increase of at least 50% in the post-vaccination sample as compared to baseline [i.e. ((Post-vac minus baseline)/baseline)100% \geq 50%]
 - If the baseline value is less or equal to 50 EU then an increase of at least 25 EU in the post-vaccination sample as compared to baseline [i.e. (post-vac minus baseline) \geq 25 EU]
- c. Number and percentage of subjects with titers post vaccination concentration \geq 121 for anti-LPS *S. sonnei* at 28 days after 1st vaccination and 28 days after 2nd vaccination
 - d. IgG Geometric mean concentrations (GMCs) pre-vaccination (Day 1), 28 days after 1st vaccination and 28 days after 2nd vaccination by antibody titer at baseline (i.e., above vs. below the assay detection limit), as determined by Enzyme-linked Immunosorbent Assay (ELISA), and applicable geometric mean ratios between post- and pre-vaccination samples.
 - e. Number and percentage of subjects with seroresponse for anti- LPS *S. sonnei* at 28 days after 1st vaccination and 28 days after 2nd vaccination by antibody titer at baseline (i.e., above vs. below the assay detection limit).

A post vaccination concentration \geq 121 anti-LPS serum IgG units in the SBVGH ELISA corresponds to a titer of 1:800 in the ELISA method used by Cohen et al. (Cohen et al., 1989). This antibody level is the median antibody concentration of a set of 87 convalescent subjects previously infected by *S. sonnei*. The value of 121 anti-LPS serum IgG units in the Novartis SBVGH ELISA was determined by calibration against the

Cohen ELISA (i.e., the SBVGH standard serum was tested in Cohen's lab using the Cohen's methodology)

8.1.3 Exploratory Endpoint(s)

8.1.3.1 Exploratory Safety Endpoint(s)

The study does not have exploratory safety endpoint(s).

8.1.3.2 Exploratory Efficacy Endpoint(s)

The study does not have exploratory efficacy endpoint(s).

8.1.3.3 Exploratory Immunogenicity Endpoint(s)

Other assays (including serum secretory IgA) might be done to further characterize the immune response to the study vaccine.

8.2 Success Criteria

This is a Phase 2a trial and there is no pre-defined success criterion.

8.2.1 Success Criteria for Primary Objective(s)

Not applicable.

8.2.1.1 Success Criteria for Primary Safety Objective(s)

Not applicable.

8.2.1.2 Success Criteria for Primary Efficacy Objective(s)

Not applicable.

8.2.1.3 Success Criteria for Primary Immunogenicity Objective(s)

Not applicable. The study does not have primary immunogenicity endpoint(s).

8.2.2 Success Criteria for Secondary Objective(s)

Not applicable.

8.2.2.1 Success Criteria for Secondary Safety Objective(s)

Not applicable. The study doesn't have secondary safety objectives.

8.2.2.2 Success Criteria for Secondary Efficacy Objective(s)

Not applicable. The study doesn't have secondary efficacy objectives.

8.2.2.3 Success Criteria for Secondary Immunogenicity Objective(s)

This is a Phase 2a trial and there are no pre-defined success criteria for secondary immunogenicity objectives.

8.3 Analysis Sets

8.3.1 All Enrolled Set

All screened subjects who provide informed consent and provide demographic and/or baseline screening assessments, regardless of the subject's randomization and treatment status in the study and received a Subject ID (where the first two digits identify the site number)

8.3.2 All Exposed Set

All subjects in the enrolled set who receive a study vaccination.

8.3.3 Safety Set

Solicited Safety Set (solicited local and systemic adverse events and other solicited adverse events)

All subjects in the Exposed Set with any solicited adverse event data and/or indicators of solicited adverse events (e.g., use of analgesics/antipyretics).

Unsolicited Safety Set (unsolicited adverse events)

All subjects in the Exposed Set with unsolicited adverse event data.

Overall Safety Set

All subjects who are in the Solicited Safety Set and/or Unsolicited Safety Set.

Subjects will be analyzed as "treated" (i.e., according to the vaccine a subject received, rather than the vaccine to which the subject may have been randomized).

8.3.4 Full Analysis Set (FAS) Immunogenicity Set

Full Analysis Set Immunogenicity

All subjects in the All Enrolled Set who are randomized, receive at least one study vaccination and provide immunogenicity data at data at relevant time points.

- Received at least one study vaccination and provide immunogenicity data 28 days after 1st vaccination. (FAS 1 - day 28 after 1st vaccination)
- Received at least one study vaccination and provide immunogenicity data 28 days after 2nd vaccination. (FAS 2 - day 28 after 2nd vaccination)

In case of vaccination error, subjects in the FAS sets will be analyzed “as randomized” (i.e., according to the vaccine a subject was designated to receive, which may be different from the vaccine the subject actually received).

8.3.5 Per Protocol (PP) Set Immunogenicity Set

All subjects in the FAS Immunogenicity who:

- Correctly receive the vaccine (i.e., receive the vaccine to which the subjects is randomized and at the scheduled time points).
- Have no protocol deviations leading to exclusion (see [section 8.3.8, Protocol Deviations](#)) as defined prior to unblinding.
- Are not excluded due to other reasons defined prior to unblinding or analysis (see [section 8.3.8, Protocol Deviations](#))

PPS are subsets of FAS and should be always defined even if the objectives do not require it.

Examples for subjects excluded due to other reasons than protocol deviations are:

- Subjects who withdrew informed consent.

8.3.6 Other Analysis Sets

Modified FAS

The differences between FAS and Modified FAS are:

- Subjects who received the same wrong vaccine at the two vaccinations will be analyzed in the vaccine the subject actually received (in the FAS they will be analyzed according to the vaccine the subject was designed to receive)
- If a subject didn't receive the vaccination but a blood sample after the vaccination visit (where subject didn't receive the vaccination) was collected then subject will be excluded from the immunogenicity analysis for all visits after the vaccination visit.

8.3.7 Subgroups

Not applicable.

8.3.8 Protocol Deviations

A protocol deviation is any change, divergence, or departure from the study design or procedures of a study protocol. A protocol deviation may be a reason to remove data from an analysis set at the time of analysis. CSR-reportable protocol deviations will be defined as exclusionary from the analysis according to protocol objectives and endpoints, which will be specified in the statistical analysis plan. In some cases exclusion of data may be due to a reason other than a protocol deviation, e.g. early termination.

8.4 Statistical Analysis Plan

8.4.1 Analysis of Demographic and Baseline Characteristics

Descriptive statistics (mean, standard deviation, median, minimum and maximum) for, age, height and weight and BMI at enrolment will be calculated overall and by vaccine group.

Distributions of subjects by sex and ethnic origin will be summarized overall and by vaccine group.

8.4.2 Analysis of Primary Objective(s)

8.4.2.1 Analysis of Primary Safety Objective(s)

The analysis of safety assessments in this study will include summaries of the following categories of safety data collected for each subject:

- Vaccine exposure.
- Solicited local and systemic adverse events.
- Unsolicited adverse events.

- Clinical Laboratory Investigations.

8.4.2.1.1 Analysis of Extent of Exposure

The frequencies and percentages of subjects with vaccinations will be summarized overall and by vaccine group. Data will be tabulated for the All Enrolled Set.

8.4.2.1.2 Analysis of Solicited Local, Systemic and Other Adverse Events

All solicited adverse events will be summarized according to defined severity grading scales.

Frequencies and percentages of subjects experiencing each adverse event will be presented for each symptom severity. Summary tables showing the occurrence of any local or systemic adverse event overall and at each time point will also be presented.

Post-vaccination solicited adverse events reported from day 1 to day 7 will be summarized for the intervals day 1-3, day 4-7, day 1-7 by maximal severity and by vaccine group. The severity of solicited local adverse events, including injection-site erythema and induration will be summarized according to categories based on linear measurement: 25 to 50 mm, 51 to 100 mm, >100mm.

Injection site pain and systemic adverse events (except fever) occurring up to 7 days after each vaccination will be summarized according to “mild”, “moderate” or “severe”.

Each solicited local and systemic adverse event will also be further summarized as “none” versus “any”.

Implausible measurements (for further definition see statistical analysis plan) will be left out of the analysis.

Use of antipyretics and analgesics will be summarized by frequency by type of use (prophylactic versus treatment) and percentage of subjects reporting use.

Body temperature will be summarized by 0.5 °C increments from 36.0 °C up to ≥ 40 °C and will be broken down according by route of measurement.

8.4.2.1.3 Analysis of Unsolicited Adverse Events

This analysis applies to all adverse events occurring during the study, judged either as probably related, possibly related, or not related to vaccination by the investigator, recorded in AE CRF, with a start date on or after the date of first vaccination. AE starting prior to the first vaccination will only be listed. The original verbatim terms used by

investigators to identify adverse events in the CRFs will be mapped to preferred terms using the MedDRA dictionary. The adverse events will then be grouped by MedDRA preferred terms into frequency tables according to system organ class (SOC).

All reported adverse events, as well as adverse events judged by the investigator as at least possibly related to study vaccine, will be summarized according to SOC and preferred term within SOC. These summaries will be presented by vaccination group. When an adverse event occurs more than once for a subject, the maximal severity and strongest relationship to the vaccine group will be counted.

Separate summaries will be produced for the following categories:

- Serious adverse events and adverse events of special interest.
- Adverse events that are possibly or probably related to vaccine.
- Adverse event leading to withdrawal.

Data listings of all adverse events will be provided by subject. In addition, adverse events in the categories above will be provided as listed data.

8.4.2.1.4 Analysis of Safety Laboratory Values

The investigator must assess all safety laboratory results ([see section 7.1.7](#)). Clinically significant modifications in blood chemistry, hematology, and urinalysis test values will be assessed by medical judgment based on interpretation of deviations from the institution's normal values.

All laboratory safety data will be analyzed descriptively by vaccine group. Safety laboratory data will be shown in a 3 x 3 table by visit using categorization of laboratory values of hematological and haematochemical blood tests and urinalysis according to institutional normal reference range (below, within, above).

8.4.2.2 Analysis of Primary Efficacy Objective(s)

This study does not include primary efficacy objectives.

8.4.2.2.1 Statistical Hypotheses

Not applicable.

8.4.2.2.2 Analysis Sets

Not applicable.

8.4.2.2.3 Statistical Methods

Not applicable.

8.4.2.3 Analysis of Primary Immunogenicity Objective(s)

This study does not have primary immunogenicity objectives.

8.4.2.3.1 Statistical Hypotheses

Not applicable.

8.4.2.3.2 Analysis Sets

Not applicable.

8.4.2.3.3 Statistical Methods

Not applicable.

8.4.3 Analysis of Secondary Objective(s)

8.4.3.1 Analysis of Secondary Safety Objective(s)

This study does not have secondary safety objectives.

8.4.3.1.1 Analysis of Extent of Exposure

Not applicable.

8.4.3.1.2 Analysis of Solicited Local, Systemic and Other Adverse Events

Not applicable.

8.4.3.1.3 Analysis of Unsolicited Adverse Events

Not applicable.

8.4.3.1.4 Statistical Hypotheses

Not applicable.

8.4.3.1.5 Analysis Sets

Not applicable.

8.4.3.1.6 Statistical Methods

Not applicable.

8.4.3.2 Analysis of Secondary Efficacy Objective(s)

This study does not have secondary efficacy objectives.

8.4.3.2.1 Statistical Hypotheses

Not applicable.

8.4.3.2.2 Analysis Sets

Not applicable.

8.4.3.2.3 Statistical Methods

Not applicable.

8.4.3.3 Analysis of Secondary Immunogenicity Objective(s)

8.4.3.3.1 Statistical Hypotheses

This Phase 2a safety and immunogenicity study is aimed to descriptively evaluate the safety and immunogenicity profiles of the study vaccines. No specific hypotheses are tested in this study.

8.4.3.3.2 Analysis Sets

The modified FAS will be the primary analysis set for the immunogenicity objective.

8.4.3.3.3 Statistical Methods

Analysis of continuous variables

The ELISA concentrations will be logarithmically transformed (base10) (to fulfil the normal distribution assumption). GMC will be calculated, with their associated two-sided 95% CIs, by exponentiating the corresponding log-transformed means and their 95% CI.

Additionally, within-subject GMRs will be computed for GMTs/GMCs at one month after first and second vaccination versus baseline (day 1). The GMRs and 95% CIs will be constructed by exponentiating the mean within-subject differences in log-transformed titers and the corresponding 95% CIs.

Analysis of binary variables

The number and percentages of subjects with seroresponse from baseline and with high antibody level after vaccination (i.e. post vaccination antibody level ≥ 121 IgG units in the SBVGH ELISA), will be summarized. Two-sided 95% Clopper-Pearson CIs for the percentages will be computed.

Titers below the limit of detection will be set to half that limit for the purposes of analysis. Missing values of immunogenicity will be excluded from analyses (i.e. complete-case analysis) since they are considered missing completely at random, i.e. not informative and with no impact on inferences.

8.4.4 Analysis of Exploratory Objectives

Other assays, including serum secretory IgA, might be done to further characterize the immune response to the study vaccine. The analysis will be described in the statistical analysis plan.

8.4.4.1 Analysis of Exploratory Safety Objective(s)

Not applicable.

8.4.4.2 Analysis of Exploratory Efficacy Objective(s)

Not applicable.

8.4.4.3 Analysis of Exploratory Immunogenicity Objective(s)

Not applicable.

8.5 Sample Size and Power Considerations of Primary Objectives

No formal statistical sample size and power computations are performed since the objectives of the study are to descriptively assess the immunogenicity and safety of the investigational vaccine.

8.6 Interim Analysis

No interim analysis of data from this study is planned.

9. SOURCE DOCUMENTATION, STUDY MONITORING AND AUDITING

Study monitoring and auditing will be standardized and performed in accordance with the Sponsor's or delegated contract research organization's (CRO) standard operating procedures and applicable regulatory requirements (e.g., FDA, EMA, and ICH guidelines).

Prior to enrolment of the first study subject, SBVGH or delegate will train investigators and/or their study staff on the study protocol, all applicable study procedures, documentation practices and all electronic systems. CRFs supplied by the Sponsor must be completed for each enrolled subject (see [section 8.3.1, All Enrolled Set](#) for definition of enrolled subject). Documentation of screened but not enrolled subjects must be maintained at the site and made available for review by the site monitor. Data and documents will be checked by the Sponsor and/or monitor.

9.1 Source Documentation

Prior to the start of the study, the site staff participating in the study conduct will be instructed on what documents will be required for review as source documents. The kinds of documents that will serve as source documents will be agreed between Sponsor or delegate and investigator and designees and specified in the SDA prior to subject enrolment.

In addition, source documentation **must** include all of the following: subject identification (on each page), eligibility and participation, proper informed consent procedures, dates of visits, adherence to protocol procedures, adequate reporting and follow-up of adverse events, documentation of prior/concomitant medication/vaccines, study vaccine receipt/dispensing/return records, study vaccine administration information, any data collected by a conversation with the subject and date of completion and reason.

The subject must also allow access to the subject's medical records. Each must be informed of this prior to the start of the study and consent for access to medical records may be required in accordance with local regulations.

All safety data reported by subjects must be written down in source documents prior to entry of the data into CRFs. If there are multiple sources of information (e.g., verbal report of the subject, telephone contact details, medical chart) supporting the diagnosis of an adverse event, these sources must be identified in the source documents, discrepancies between sources clarified, the ultimate diagnosis must be justified and written in the source documents, and this diagnosis must be captured in the Adverse Event CRF (AE CRF). The AE CRF must also capture which source(s) of information were used to determine the adverse event (e.g., subject recall, medical chart).

9.2 Study Monitoring, Auditing and Source Data Verification

Prior to enrolment of the first study subject, SBVGH or its designee (e.g., a CRO) will develop a Clinical Monitoring Plan (or comparable documentation) to specify how centralized and/or on-site monitoring, including clinical specimens reconciliation, will be performed for the study. Study progress will be monitored by SBVGH or its designee as frequently as necessary to ensure:

- that the rights and well-being of human subjects are protected,
- the reported study data are accurate, complete, and verifiable from the source documents and
- the conduct of the study is in compliance with the current approved protocol/amendment(s), GCP and applicable regulatory requirements.

Contact details for the SBVGH team or its designee involved in study monitoring will be provided to the investigator. Study data recorded on CRFs will be verified by checking the CRF entries against source documents in order to ensure data completeness and accuracy as required by study protocol except for those parameters which are specifically described in [section 7, Assessment](#) being entered directly into the EDC system.

Data verification may also be performed through a centralized review of data (e.g., checking for outliers or other anomalies). Additional documents such as the investigator site file, pharmacy records, and informed consent documentation must also be available for review if requested. Arrangements for monitoring visits will be made in advance in accordance with the monitoring plan, except in case of emergency.

The investigator and/or site staff must make source documents of subjects enrolled in this study available for inspection by SBVGH or its representative at the time of each monitoring visit and Sponsor audits, when applicable. These documents must also be available for inspection, verification and copying, as required by regulations, by officials of the regulatory health authorities (e.g., FDA, EMA and others) and/or ECs/IRBs. The investigator and study site staff must comply with applicable privacy, data protection and medical confidentiality laws for use and disclosure of information related to the study and enrolled subjects.

10. DATA MANAGEMENT

10.1 Data Entry and Management

In this study, all clinical data (including, but not limited to, AE/SAEs, concomitant medications, medical history, and physical assessments), safety data, and immunogenicity data will be entered onto case report forms (CRFs) in a timely fashion by the investigator and/or the investigator's dedicated site staff. Data entered onto CRFs are stored on a secure website. The data collected on this secure website are assimilated into an electronic data capture (EDC) system, which is compliant with Title 21 Part 11 policies of the Code of Federal Regulations ([FDA 1997](#)). The data system includes password protection and internal quality checks. The EDC system will be designed and validated by the Sponsor prior to activation for data entry by sites. The investigator or designated delegate must review data entered and electronically sign the CRFs to verify their accuracy.

Access to the EDC system for data entry or review will require training and distinct individual access code assignments to those site staff members who will be entering study data and those involved in study oversight who may review study data. Data are collected within the EDC system, to which the Sponsor and site monitors have exclusively "read only" access.

10.2 Data Clarification

As part of the conduct of the trial, the Sponsor may have questions about the data entered by the site, referred to as queries. The monitors and the Sponsor are the only parties that can generate a query. All corrections and clarifications will be entered into the EDC system and will be identified by the person entering the information, the reason for the change, as well as the time of the changes made. If changes are made to a previously and electronically signed CRF, the investigator must confirm and endorse the changes.

10.3 Data Protection

SBVGH respects the subjects' rights to privacy and will ensure the confidentiality of their medical information in accordance with all applicable laws and regulations.

11. RECORD RETENTION

Investigators must retain all study records required by SBVGH and by the applicable regulations in a secure and safe facility. The investigator must consult a SBVGH representative before disposal of any study records, and must notify the Sponsor of any change in the location, disposition, or custody of the study files. Essential documents must be retained for 15 years. “Essential documents” are defined as documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced. These documents should be retained for a longer period, however, if required by the applicable national regulatory or institutional requirements.

These principles of record retention will also be applied to the storage of laboratory samples, provided that the integrity of the stored sample permits testing.

12. USE OF INFORMATION AND PUBLICATION

SBVGH assures that the key design elements of this protocol will be posted in a publicly accessible database such as clinicaltrials.gov, and in compliance with current regulations.

SBVGH also assures that key results of this clinical study will be posted in a publicly accessible database within the required time-frame from the end of study as defined in [section 3.9, End of Study](#).

In accordance with standard editorial, ethical practices and current guidelines of Good Publication Practice ([Graf 2009](#)), SBVGH will generally support publication of multicenter studies only in their entirety and not as individual center data. In this case, a coordinating investigator will be designated by mutual agreement prior to the start of the study. The coordinating investigator will also sign the clinical study report on behalf of the principal investigators ([CPMP/EWP/2747/00](#)). Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements. Any formal publication of the study in which contribution of SBVGH personnel exceeded that of conventional monitoring will be considered as a joint publication by the investigator and the appropriate SBVGH personnel.

SBVGH must be notified of any intent to publish data collected from the study and prior approval from SBVGH must be obtained prior to submission for publication.

13. ETHICAL CONSIDERATIONS

13.1 Regulatory and Ethical Compliance

The study will be conducted in compliance with the protocol, GCP and applicable regulatory requirement(s).

This clinical study was designed and shall be implemented and reported in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations and with the ethical principles laid down in the Declaration of Helsinki ([European Council 2001](#), [US Code of Federal Regulations](#), [ICH 1997](#)).

13.2 Informed Consent Procedures

Eligible subjects may only be included in the study after providing written informed consent, as described in [section 5.1.1, Informed Consent/Assent](#). Before the start of the study, the investigator will have the informed consent and any other materials that will be provided to the subjects reviewed and approved by the IRB/EC. This review and approval will be documented and stored with other study documents. The investigator or designee must fully inform the subject or legal guardian of all pertinent aspects of the study. A copy of the written informed consent will be given to the subject or the designee. The subject/designee must be allowed ample time to ask about the details of the study and to make a decision as to whether or not to participate in the study. The subject **must** sign the consent form indicating their agreement to participate in the study before any study-related procedures are conducted. If the subject is unable to read and write, a witness must be present during the informed consent discussion and at the time of informed consent signature.

Prior to the start of the study, SBVGH will provide to investigators a proposed informed consent form that complies with the ICH GCP guideline and regulatory requirements and is considered appropriate for this study. Any changes to the proposed consent form suggested by the investigator must be agreed to by SBVGH before submission to the IRB/EC and a copy of the approved version must be provided to the SBVGH monitor after IRB/EC approval.

Women of childbearing potential should be informed that taking the study medication may involve unknown risks to the fetus if pregnancy were to occur during the study and agree that in order to participate in the study they must adhere to the contraception requirements indicated in the protocol for the duration of the study. If case of doubts on the ability of a subject to adhere to these requirements, that subject should not be allowed in the study.

13.3 Responsibilities of the Investigator and IRB/EC

The protocol and the proposed informed consent form must be reviewed and approved by a properly constituted IRB/EC before study start. Properly constituted IRB/EC is defined in ICH Guideline for Good Clinical Practice E6 (R1), Section 3 ([ICH 1997](#)). A signed and dated statement that the protocol and informed consent have been approved by the IRB/EC must be given to SBVGH before study initiation. Prior to study start and at any time the protocol is amended during study conduct, the investigator is required to sign a protocol signature page confirming his/her agreement to conduct the study in accordance with these documents and all of the instructions and procedures found in this protocol and to give access to all relevant data and records to SBVGH monitors, auditors, SBVGH Clinical Quality Assurance representatives, designated agents of SBVGH, IRBs/ECs, and regulatory authorities as required. If an inspection of the clinical site is requested by a regulatory authority, the investigator must inform SBVGH immediately that this request has been made.

The investigator also responsible for the following:

- Maintaining a list of appropriately qualified persons to whom the investigator has delegated significant study-related duties.
- Demonstrating the capability of recruiting the required number of suitable subjects within the recruitment period.
- Demonstrating sufficient time and staffing to properly conduct and complete the study within the agreed study period.
- Ensuring that all persons assisting with the study are adequately informed about the protocol, the investigational product(s), and their study-related duties and functions
- Ensuring that appropriately trained health care professionals are responsible for all study-related medical decisions and for ensuring appropriate medical care of subjects experiencing any adverse event related to the study.

The investigator should not implement any deviation from, or changes of the protocol without agreement by the Sponsor and prior review and documented approval/favourable opinion from the IRB/IEC of an amendment, except where necessary to eliminate an immediate hazard(s) to study subjects, or when the change(s) involves only logistical or administrative aspects of the study (e.g., change in monitor(s), change of telephone number(s)). In addition, the investigator, or person designated by the investigator, should document and explain any deviation from the approved protocol.

The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to study subjects without prior IRB/IEC

approval/favourable opinion. As soon as possible, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment(s) should be submitted:

- (a) to the IRB/IEC for review and approval/favourable opinion,
- (b) to the Sponsor for agreement and, if required,
- (c) to the regulatory authority(ies).

13.4 Protocol Amendments

An amendment is a written description of change(s) to or formal clarification of a study protocol which may impact on the conduct of the clinical study, potential benefit of the clinical study, or may affect subject safety, including changes of study objectives, study design, subject population, sample sizes, study procedures, or significant administrative aspects. An administrative change of a study protocol is a minor correction or clarification that has no significant impact on the way the clinical study is to be conducted and no effect on subject safety (e.g., change of telephone number(s), logistical changes). Protocol amendments must be approved by SBVGH, health authorities where required, and the IRB/EC. In cases when the amendment is required in order to protect the subject safety, the amendment can be implemented prior to IRB/EC approval. Notwithstanding, the need for formal approval of a protocol amendment, the investigator is expected to take any immediate action required for the safety of any subject included in this study, even if this action represents a deviation from the protocol. In such cases, SBVGH should be notified of this action, the IRB/EC at the study site, and, if required by local regulations, the relevant health authority) should be informed within 10 working days.

14. REFERENCE LIST

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Protocol Amendment Summary Form

Protocol #:	H03_04TP		
Current Protocol Version # and date:	Version 1.0 of 28 July 2015		
Protocol Title:	A Phase 2a, Observer Blind, Randomized, Controlled, Single Center Study to Evaluate the Safety, Reactogenicity, and Immunogenicity of 2 Doses of the SBVGH 1790GAHB Vaccine against Shigella sonnei, Administered Intramuscularly in Adult Subjects from a Country Endemic for Shigellosis.		
Proposed Amendment Protocol # and date:	Amendment 1 of 03 November 2015 Protocol Version 2.0 of 04 November 2015		
Protocol Amendment Summary Form Author:	PPD [redacted]	/ PPD [redacted]	PPD [redacted]
Type of amendment:	<input checked="" type="checkbox"/> Non-substantial <input type="checkbox"/> Substantial Regulatory Affairs signature: 05. Nov. 2015		
Other revisions required?			
Case Report Form	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No	<input type="checkbox"/> Not Applicable
Informed Consent Form	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No	<input type="checkbox"/> Not Applicable
Statistical Analysis Plan	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No	<input type="checkbox"/> Not Applicable
Study Manual	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No	<input type="checkbox"/> Not Applicable
Other (specify):	<input type="checkbox"/> Yes	<input type="checkbox"/> No	<input type="checkbox"/> Not Applicable

Amendment Rationale:

The purposes of Amendment 1 to H03_04TP study Protocol v. 1.0 of 28 July 2015 are the following:

- 1) Clarify how randomization is performed.
- 2) As requested by the Scientific and Ethics Review Unit (SERU):
 - a. Clarify for how long sera samples and left over samples after testing will be archived at clinical site and sponsor laboratories and provide a clear justification for this archival.

- b. Clarify the nature of study-related and non-study related sample testing / future research that may be done after study conclusion.
 - c. Indicate whether participants will be contacted in the future on results obtained from future sample testing / research.
 - d. Clarify the reimbursement provided to the study participants per visit.
- 3) Change the name of the sponsor: i.e. replace “Sclavo Behring Vaccines Institute for Global Health (SBVGH), a GSK company” with the new name “GSK Vaccines Institute for Global Health (GVGH)”. This is an administrative change requested following sponsor’s name change.

Proposed Changes:

PAGE/SECTION <i>Current page and section numbers being changed</i>	CURRENT <i>Language currently in use in protocol</i>	PROPOSED CHANGE <i>Language proposed</i>	JUSTIFICATION <i>State specific reason for the change or refer to rationale</i>
Page 29 Section 3. STUDY DESIGN Sub-section 3.1 Overview of Study Design	Within each group, in an observer-blind fashion, subjects will be randomized to receive two intramuscular vaccinations, four weeks apart.	Subjects will be randomized to one of the three vaccine groups and all subjects will receive two intramuscular vaccinations, four weeks apart.	To better describe how randomization is performed.
Page 33 Section 3.5 Collection of Clinical Specimens	Shipment to the laboratories for analysis will be performed according to sites guidelines provided by the sponsor.	One aliquot of serum will be maintained at the KEMRI-Wellcome Trust laboratory. The other aliquots will be shipped according to guidelines provided by the sponsor to the laboratories for analysis.	To specify that one serum aliquot will be maintained at the clinical site laboratoy.
Page 33	Not applicable (new text	Aliquots of sera will be	As requested by EC (SERU).

<p>Section 3.5 Collection of Clinical Specimens</p>	<p>added)</p>	<p>archived for 15 years both at KEMRI-WT laboratory in Kilifi and GSK laboratory in Marburg (Germany) for future research on immunogenicity of the Shigella vaccine. Study-related future research may include additional evaluation of immunogenicity on Shigella sonnei (i.e. IgM, IgA against the O antigen or IgG against other antigens of Shigella sonnei). Non-study related future research may include serology on other Shigella serotypes and other bacteria causing infectious diseases in developing countries (e.g. Salmonella Typhi, Salmonella Paratyphi, non-typhoidal Salmonella, meningitis) to inform development of other vaccines relevant to the populations in developing countries. It may not be possible to contact individual participants in the future in order to disseminate results obtained from research conducted on</p>	
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		archived samples. However, these results, if relevant, will be published in open access peer-reviewed journals and disseminated to Kilifi county leaders and the community. An archival time of 15 years should cover the entire vaccine development time, including the possibility to address potential questions from regulators during the registration time.	
Page 83 Section 13. ETHICAL CONSIDERATIONS Sub-section 13.1 Regulatory and Ethical Compliance	Not applicable (new text added)	For each scheduled study visit (Screening visit and Visit 1 to 5) subjects will be reimbursed Kenyan Shillings (KSh) 1,000 per visit as follows: - KSh 500 for daily lost wages and time spent at study clinic; - KSh 300 for the cost of transportation; - KSh 200 for food.	As requested by EC (SERU)
Study title and throughout the whole protocol	Sclavo Behring Vaccines Institute for Global Health (SBVGH)	GSK Vaccines Institute for Global Health (GVGH)	Administrative change

**Protocol Amendment Summary Form**

Study #:	H03_04TP
Current Protocol Version # and date:	Version 2.0 of 04 November 2015
Protocol Title:	A Phase 2a, Observer Blind, Randomized, Controlled, Single Center Study To Evaluate The Safety, Reactogenicity And Immunogenicity Of 2 Doses Of The GVGH 1790GAHB Vaccine Against Shigella Sonnei, Administered Intramuscularly In Adult Subjects From A Country Endemic For Shigellosis
Amendment # and date:	Amendment # 2 of 25 May 2016
Amended Protocol # and date:	Version 3.0 of 27 May 2016
Protocol Amendment Summary Form Author:	PPD [redacted] / PPD [redacted]
Type of amendment:	<input type="checkbox"/> Non-substantial <input checked="" type="checkbox"/> Substantial PPD [redacted] Regulatory Affairs signature: PPD [redacted] 31 May 2016

Other revisions required?			
Case Report Form	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No	<input type="checkbox"/> Not Applicable
Informed Consent Form	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No	<input type="checkbox"/> Not Applicable
Statistical Analysis Plan	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No	<input type="checkbox"/> Not Applicable
Study Manual	<input type="checkbox"/> Yes	<input checked="" type="checkbox"/> No	<input type="checkbox"/> Not Applicable
Other (specify): Study-specific workbook	<input checked="" type="checkbox"/> Yes	<input type="checkbox"/> No	<input type="checkbox"/> Not Applicable

Amendment Rationale:

The purposes of Amendment 2 (classified as substantial) to H03_04TP study Protocol V2.0 of 04 November 2015 are described below:

The Development Core Safety Information (DCSI) issued on 25/05/16 includes the following precautions for 1790GAHB Shigella vaccine:

As precautionary measures future clinical trial protocols with particular attention to paediatric population will include monitoring of neutrophil counts (i.e., full blood counts with differential) at baseline and approximately 7 days post each scheduled vaccination, and periodically thereafter until resolution in case of neutropenia, and any subjects with neutropenic fever or ANC < 500/mm³ will be



discontinued from further study participation. Additionally, exclusion criteria should include, subjects with a low baseline neutrophil count (i.e., below normal ranges), previous history of Benign Ethnic Neutropenia or drug related neutropenia as reported at screening visit during review of medical history, and any subjects who have or are likely to require concomitant treatment with neutropenic agents. Finally, in this study and future studies, any new onset of neutropenia occurring after vaccination will be considered as an Adverse Event of Special Interest (AESI).

For definition of the severity of neutropenia episodes, the following grading system will be adopted:

Adverse events of special interest*	Grade 1	Grade 2	Grade 3	Grade 4
Neutropenia	ANC < LLN - >1.5 x 10 ⁹ /L	ANC <1.5 – 1.0 x 10 ⁹ /L	ANC <1.0 – 0.5 x 10 ⁹ /L	ANC <0.5 x 10 ⁹ /L

*AESI grading according to Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 of 28May2009.

Implications:

Although most of the above precautions are already part of the approved protocol H03_04TP (V2.0), the following two are not included:

- 1) Post-vaccination Neutropenia is not considered as an Adverse Event of Special Interest (AESI) in V2.0 of the protocol
- 2) Exclusion criteria do not include subjects with a previous history of Benign Ethnic Neutropenia or drug related neutropenia and subjects who have or are likely to require concomitant treatment with neutropenic agents

Therefore the purpose of amendment 2 is ensure in amended protocol V3.0 neutropenia is considered an AESI (in addition to reactive arthritis) and also that exclusion criteria will include subjects with a previous history of Benign Ethnic Neutropenia or drug-related neutropenia, and subjects who have or are likely to require concomitant treatment with neutropenic agents.

No change is necessary in the study procedures and no other document (except for the study-specific workbook) should be modified including ICF.

**Proposed Changes:**

PAGE/SECTION <i>Current page and section numbers being changed</i>	CURRENT <i>Language currently in use in protocol</i>	PROPOSED CHANGE <i>Language proposed</i>	JUSTIFICATION <i>State specific reason for the change or refer to rationale</i>
Page 11 PROTOCOL SYNOPSIS / Study Design	Reactive arthritis will be collected and analyzed as an AESI for this study.	Reactive arthritis or neutropenia will be collected and analyzed as AESIs for this study.	To implement the precautions for the 1790GAHB Shigella vaccine as recommended in the Development Core Safety Information (DCSI).
Page 15 PROTOCOL SYNOPSIS / Primary Endpoint(s)	Number and percentage of subjects with reported reactive arthritis (AESIs).	Number and percentage of subjects with reported reactive arthritis or neutropenia (defined AESIs).	To implement the precautions for the 1790GAHB Shigella vaccine as recommended in the Development Core Safety Information (DCSI).
Page 27 1. BACKGROUND AND RATIONALE / 1.1 Background	During the two phase 1 trials three subjects of African descent experienced a transient and clinically asymptomatic decrease of circulating neutrophils (two episodes were graded as severe and one as moderate) that was finally classified as "benign ethnic neutropenia".	During the two phase 1 trials two subjects of African descent experienced a transient and clinically asymptomatic decrease of circulating neutrophils of grade 3 (severe) that was finally classified as "benign ethnic neutropenia". In addition, six more subjects experienced transient and clinically asymptomatic grade 2 (moderate) neutropenia.	Rewording to make it consistent to the information in the Investigator's Brochure
Page 30 3. STUDY DESIGN / 3.1 Overview of Study	Any unsolicited AE, serious adverse events (SAEs), all AEs leading to	Any unsolicited AE, serious adverse events (SAEs), all AEs leading to	To implement the precautions for the 1790GAHB Shigella vaccine as recommended in the Development Core Safety Information (DCSI).



Design	vaccine/study withdrawal, and reactive arthritis (adverse event of special interest (AESI)) and all concomitant medications associated with those events, will be collected for the entire study and recorded in the subject's source document.	vaccine/study withdrawal, reactive arthritis and neutropenia (adverse event of special interest (AESI)) and all concomitant medications associated with those events, will be collected for the entire study and recorded in the subject's source document.	
Page 34-35 3. STUDY DESIGN / 3.7 Procedures related to neutropenia	During the two phase 1 trials with 1790GAHB, three subjects of African descent experienced a transient and clinically asymptomatic decrease of circulating neutrophils (two graded as severe and one as moderate) that was finally classified as "benign ethnic neutropenia".	During the two phase 1 trials with 1790GAHB, two subjects of African descent experienced a transient and clinically asymptomatic decrease of circulating neutrophils of grade 3 (severe) that was finally classified as "benign ethnic neutropenia". In addition, six more subjects experienced transient and clinically asymptomatic grade 2 (moderate) neutropenia.	Rewording to make it consistent to the information in the Investigator's Brochure
Page 36 3. STUDY DESIGN / 3.7 Procedures related to neutropenia	N/A	For classification and grading of severity of neutropenia for the first subset of 18 subjects, the criteria of Common Terminology Criteria for Adverse Events (CTCAE) issued by US Department of	Clarification of criteria to be adopted for grading of neutropenia during the trial.



		Health and Human Services (version 4.3; 2010) will be adopted. In case DSMB endorses the use of local ranges of normality for the second subset of 54	
		subjects, the grading will be modified based on input from DSMB.	
Page 36 3. STUDY DESIGN / 3.7 Procedures related to neutropenia	N/A	Finally, all cases of neutropenia regardless of intensity will be considered as Adverse Events of Special Interest (AESI) from a reporting perspective.	To implement the precautions for the 1790GAHB Shigella vaccine as recommended in the Development Core Safety Information (DCSI).
Page 42 4. SELECTION OF STUDY POPULATION/ 4.2 Exclusion Criteria	N/A	22. Individuals with a previous history of Benign Ethnic Neutropenia or drug related neutropenia 23. Individuals who have or are likely to require concomitant treatment with neutropenic agents	To implement the precautions for the 1790GAHB Shigella vaccine as recommended in the Development Core Safety Information (DCSI).
Page 63 7. ASSESSMENTS / 7.1.4.1 Adverse Events of Special Interest	N/A	Neutropenia, a decrease of circulating neutrophils below the lower limit of the range of normality, will be collected and analyzed as an AESI for this study. To this purpose, as specified in the study procedures, subjects in this trial will have multiple	To implement the precautions for the 1790GAHB Shigella vaccine as recommended in the Development Core Safety Information (DCSI).



		blood draws for monitoring potential episodes of post vaccination neutropenia. For additional information on neutropenia, please refer to Section 3.7, Procedures Related To Neutropenia	
Page 67 8. STATISTICAL CONSIDERATIONS / 8.1.1.1 Primary Safety Endpoint(s)	e. Number and percentage of subjects with reported reactive arthritis (AESIs).	e. Number and percentage of subjects with reported reactive arthritis or neutropenia (AESIs).	To implement the precautions for the 1790GAHB Shigella vaccine as recommended in the Development Core Safety Information (DCSI).

**Protocol Amendment Summary Form**

Study #:	H03 04TP
Current Protocol Version # and date:	Version 3.0 of 27 May 2016
Protocol Title:	A Phase 2a, Observer Blind, Randomized, Controlled, Single Center Study To Evaluate The Safety, Reactogenicity And Immunogenicity Of 2 Doses Of The GVGH 1790GAHB Vaccine Against Shigella Sonnei, Administered Intramuscularly In Adult Subjects From A Country Endemic For Shigellosis
Amendment # and date:	Amendment # 3 of 02 November 2016
Amended Protocol # and date:	Version 4.0 of 03 November 2016
Protocol Amendment Summary Form Author:	PPD [REDACTED]
Type of amendment:	[X] Non-substantial [] Substantial PPD [REDACTED] Regulatory Affairs signature: [REDACTED] 11 Nov 2016

Other revisions required?			
Case Report Form	[] Yes	[X] No	[] Not Applicable
Informed Consent Form	[] Yes	[X] No	[] Not Applicable
Statistical Analysis Plan	[X] Yes	[] No	[] Not Applicable
Study Manual (Clinical Specimen Laboratory Manual)	[X] Yes	[] No	[] Not Applicable
Other (specify):	[] Yes	[X] No	[] Not Applicable



Amendment Rationale:

The purpose of Amendment #3 to H03_04TP study protocol v3.0 of 27 May 2016 (classified as non-substantial) is as follows:

- Introduce a group unblinded preliminary immunogenicity analysis and a blinded interim safety analysis for up to 45 subjects included in the study after they have completed the study. The results of these immunogenicity and safety analyses will not impact the conduct of the study, but will inform the planning of future studies with the same vaccine.
- To correct a typo to ensure consistency in the protocol.
- Include a sentence for management of IMP (preparation, possibly necessary additional labeling) at clinical site.

Implications: No changes to the study procedures are necessary and the only other documents requiring a change are the Clinical Specimen Laboratory Manual (that will be updated to include the additional sera shipment(s) as per serology plan) and the Statistical Analysis Plan. The ICF is not impacted.

**Proposed Changes:**

PAGE/SECTION <i>Current page and section numbers being changed</i>	CURRENT <i>Language currently in use in protocol</i>	PROPOSED CHANGE <i>Language proposed</i>	JUSTIFICATION <i>State specific reason for the change or refer to rationale</i>
Page 14 PROTOCOL SYNOPSIS / Study Vaccines	N/A	Bedside mixing, possibly necessary additional labelling of clinical study materials at the study site and vaccine administration will be performed according to GVGH instructions and by trained site staff. The site staff responsible for these activities will be personnel who are respectively qualified according to applicable laws and regulations. GVGH will provide specific procedures and training for these activities.	Include a sentence for management of IMP (preparation, possibly necessary additional labeling) at clinical site.
Page 17 PROTOCOL SYNOPSIS / Interim Analysis	No interim analysis will be performed.	For up to 45 subjects, once they have completed the study, a group unblinded preliminary immunogenicity analysis and a blinded interim safety analysis will be performed. Individual subject results from preliminary analyses will not be made available to site	To inform the planning of future studies with the same vaccine.



		and sponsor personnel until the end of the study. The results of these immunogenicity and safety analyses will not impact the conduct of the study, but will inform the planning of future studies with the same vaccine (see also section 8.6 of the protocol).	
Page 42 4. SELECTION OF STUDY POPULATION / 4.2 Exclusion Criteria	16. Individuals with body temperature > 38.0°C within 3 days of intended study vaccination is a reason for delay of vaccination (see section 4.3 Criteria for Delay of Vaccination for further details).	16. Individuals with body temperature ≥ 38.0°C within 3 days of intended study vaccination is a reason for delay of vaccination (see section 4.3 Criteria for Delay of Vaccination for further details).	To correct a typo and to be consistent with what is written for body temperature in section 4.3 Criteria for Delay of Vaccination .
Page 54 6.TREATMENT OF SUBJECTS / 6.3 Vaccine Preparation and Administration	N/A	Bedside mixing and vaccine administration at the study site will be performed according to GVGH instructions and by trained site staff. The site staff responsible for these activities will be personnel who are respectively qualified according to applicable laws and regulations. GVGH will provide specific procedures and training for these	Include a sentence for management of IMP (preparation, possibly necessary additional labeling) at clinical site.



		activities.	
Page 56 6.TREATMENT OF SUBJECTS / 6.6 Vaccine Supply, Labeling, Storage and Tracking	N/A	Possibly necessary additional labelling of clinical study materials at the study site will be performed according to GVGH instructions and by trained site staff who are qualified according to applicable laws and regulations.	Include a sentence for management of IMP (preparation, possibly necessary additional labeling) at clinical site.
Page 79 8.STATISTICAL CONSIDERATIONS / TREATMENT OF SUBJECTS/ 8.6 Interim Analysis	No interim analysis of data from this study is planned.	For up to 45 subjects, once they have completed the study, a group unblinded preliminary immunogenicity analysis and a blinded interim safety analysis will be performed. Individual subject results from preliminary analyses will not be made available to site and sponsor personnel until the end of the study. The results of these immunogenicity and safety analyses will not impact the conduct of the study, but will inform the planning of future studies with the same vaccine.	To inform the planning of future studies with the same vaccine.

CLINICAL STUDY PROTOCOL H03_04TP

Amendment 1 of 03 November 2015

Version 2.0 of 04 November 2015

Amendment 2 of 25 May 2016

Version 3.0 of 27 May 2016

Amendment 3 of 02 Nov 2016

Version 4.0 of 03 Nov 2016

A Phase 2a, Observer Blind, Randomized, Controlled, Single Center Study To Evaluate The Safety, Reactogenicity And Immunogenicity Of 2 Doses Of The GVGH 1790GAHB Vaccine Against *Shigella Sonnei*, Administered Intramuscularly In Adult Subjects From A Country Endemic For Shigellosis

Property of GSK Vaccines Institute for Global Health (hereafter referred to as GVGH)

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TABLE OF CONTENTS

TABLE OF CONTENTS.....	2
PROTOCOL SYNOPSIS H03_04TP	8
LIST OF ABBREVIATIONS.....	23
1. BACKGROUND AND RATIONALE	25
1.1 Background.....	26
1.2 Rationale	27
2. OBJECTIVES.....	28
2.1 Primary Objective(s).....	28
2.2 Secondary Objective(s).....	28
2.3 Exploratory Objective(s)	28
3. STUDY DESIGN	29
3.1 Overview of Study Design.....	29
3.2 Study Period.....	30
3.3 Blinding Procedures.....	30
3.4 Data Collection	31
3.4.1 Data Collected from Subjects	31
3.4.2 Tools Used for Data Collection	31
3.5 Collection of Clinical Specimens	32
3.6 Stopping/Pausing Guidelines.....	34
3.7 Procedures related to neutropenia.....	34
3.8 Data Monitoring Committee.....	36
3.9 Premature Withdrawal from Study.....	36
3.10 End of Study	39
4. SELECTION OF STUDY POPULATION.....	40
4.1 Inclusion Criteria	40
4.2 Exclusion Criteria	40
4.3 Criteria for Delay of Vaccination	42
5. STUDY PROCEDURES.....	44

5.1	Pre-vaccination Clinic Visit(s)	44
5.1.1	Informed Consent/Assent	44
5.1.2	Screening	45
5.1.3	Enrolment	46
5.1.4	Randomization	47
5.2	Vaccination Clinic Visit(s)	47
5.2.1	Post-vaccination Procedures	48
5.2.2	Post-vaccination Reminders	48
5.3	Post-vaccination Visit(s)	48
5.3.1	Follow-up Home Visit(s)	48
5.3.2	Follow-up Clinic Visit(s)	49
5.3.3	Safety Follow-up Calls	50
5.4	Unscheduled Visits	50
5.5	Study Termination Visit	50
5.5.1	Early Termination Visit	51
6.	TREATMENT OF SUBJECTS	53
6.1	Study Vaccine(s)	53
6.2	Non-Study Vaccines	54
6.3	Vaccine Preparation and Administration	54
6.4	Vaccine Administration Error or Overdose of Vaccine	55
6.5	Prior and Concomitant Medications and Vaccines	55
6.6	Vaccine Supply, Labeling, Storage and Tracking	56
7.	ASSESSMENTS	58
7.1	Safety Assessment	58
7.1.1	Solicited Adverse Events	58
7.1.2	Unsolicited Adverse Events	61
7.1.3	Evaluation of Adverse Events	61
7.1.4	Serious Adverse Events	63
7.1.4.1	Adverse Events of Special Interest	64

7.1.5	Methods for Recording Adverse Events and Serious Adverse Events	65
7.1.5.1	Post-Study Events	65
7.1.6	Pregnancies	66
7.1.7	Safety Laboratory Measurements	66
7.2	Efficacy Assessment	67
7.3	Immunogenicity Assessment	67
8.	STATISTICAL CONSIDERATIONS	68
8.1	Endpoints	68
8.1.1	Primary Endpoint(s)	68
8.1.1.1	Primary Safety Endpoint(s)	68
8.1.1.2	Primary Efficacy Endpoint(s)	68
8.1.1.3	Primary Immunogenicity Endpoint(s)	68
8.1.2	Secondary Endpoint(s)	68
8.1.2.1	Secondary Safety Endpoint(s)	68
8.1.2.2	Secondary Efficacy Endpoint(s)	68
8.1.2.3	Secondary Immunogenicity Endpoint(s)	69
8.1.3	Exploratory Endpoint(s)	70
8.1.3.1	Exploratory Safety Endpoint(s)	70
8.1.3.2	Exploratory Efficacy Endpoint(s)	70
8.1.3.3	Exploratory Immunogenicity Endpoint(s)	70
8.2	Success Criteria	70
8.2.1	Success Criteria for Primary Objective(s)	70
8.2.1.1	Success Criteria for Primary Safety Objective(s)	70
8.2.1.2	Success Criteria for Primary Efficacy Objective(s)	70
8.2.1.3	Success Criteria for Primary Immunogenicity Objective(s)	70
8.2.2	Success Criteria for Secondary Objective(s)	70
8.2.2.1	Success Criteria for Secondary Safety Objective(s)	71
8.2.2.2	Success Criteria for Secondary Efficacy Objective(s)	71
8.2.2.3	Success Criteria for Secondary Immunogenicity Objective(s)	71

8.3	Analysis Sets.....	71
8.3.1	All Enrolled Set	71
8.3.2	All Exposed Set	71
8.3.3	Safety Set.....	71
8.3.4	Full Analysis Set (FAS) Immunogenicity Set	72
8.3.5	Per Protocol (PP) Set Immunogenicity Set.....	72
8.3.6	Other Analysis Sets.....	72
8.3.7	Subgroups	73
8.3.8	Protocol Deviations	73
8.4	Statistical Analysis Plan	73
8.4.1	Analysis of Demographic and Baseline Characteristics	73
8.4.2	Analysis of Primary Objective(s)	73
8.4.2.1	Analysis of Primary Safety Objective(s)	73
8.4.2.1.1	Analysis of Extent of Exposure	74
8.4.2.1.2	Analysis of Solicited Local, Systemic and Other Adverse Events.....	74
8.4.2.1.3	Analysis of Unsolicited Adverse Events	74
8.4.2.1.4	Analysis of Safety Laboratory Values.....	75
8.4.2.2	Analysis of Primary Efficacy Objective(s).....	75
8.4.2.2.1	Statistical Hypotheses	75
8.4.2.2.2	Analysis Sets	75
8.4.2.2.3	Statistical Methods	76
8.4.2.3	Analysis of Primary Immunogenicity Objective(s).....	76
8.4.2.3.1	Statistical Hypotheses.....	76
8.4.2.3.2	Analysis Sets	76
8.4.2.3.3	Statistical Methods	76
8.4.3	Analysis of Secondary Objective(s)	76
8.4.3.1	Analysis of Secondary Safety Objective(s)	76
8.4.3.1.1	Analysis of Extent of Exposure	76
8.4.3.1.2	Analysis of Solicited Local, Systemic and Other Adverse Events.....	76

8.4.3.1.3	Analysis of Unsolicited Adverse Events	76
8.4.3.1.4	Statistical Hypotheses	76
8.4.3.1.5	Analysis Sets	77
8.4.3.1.6	Statistical Methods	77
8.4.3.2	Analysis of Secondary Efficacy Objective(s).....	77
8.4.3.2.1	Statistical Hypotheses	77
8.4.3.2.2	Analysis Sets	77
8.4.3.2.3	Statistical Methods	77
8.4.3.3	Analysis of Secondary Immunogenicity Objective(s).....	77
8.4.3.3.1	Statistical Hypotheses	77
8.4.3.3.2	Analysis Sets	77
8.4.3.3.3	Statistical Methods	77
8.4.4	Analysis of Exploratory Objectives.....	78
8.4.4.1	Analysis of Exploratory Safety Objective(s).....	78
8.4.4.2	Analysis of Exploratory Efficacy Objective(s)	78
8.4.4.3	Analysis of Exploratory Immunogenicity Objective(s).....	78
8.5	Sample Size and Power Considerations of Primary Objectives	78
8.6	Interim Analysis.....	79
9.	SOURCE DOCUMENTATION, STUDY MONITORING AND AUDITING	80
9.1	Source Documentation.....	80
9.2	Study Monitoring, Auditing and Source Data Verification.....	81
10.	DATA MANAGEMENT	82
10.1	Data Entry and Management	82
10.2	Data Clarification.....	82
10.3	Data Protection	82
11.	RECORD RETENTION	83
12.	USE OF INFORMATION AND PUBLICATION	84
13.	ETHICAL CONSIDERATIONS	85
13.1	Regulatory and Ethical Compliance	85

13.2	Informed Consent Procedures.....	85
13.3	Responsibilities of the Investigator and IRB/EC.....	86
13.4	Protocol Amendments	87
14.	REFERENCE LIST	88

PROTOCOL SYNOPSIS H03_04TP

Name of Sponsor: GSK Vaccines Institute for Global Health (GVGH)	Protocol number: H03_04TP	Generic name of study vaccine(s): <i>S. sonnei</i> 1790GAHB Vaccine
Title of Study: A Phase 2a, Observer Blind, Randomized, Controlled, Single Center Study To Evaluate The Safety, Reactogenicity And Immunogenicity Of 2 Doses Of The GVGH 1790GAHB Vaccine Against <i>Shigella Sonnei</i> , Administered Intramuscularly In Adult Subjects From A Country Endemic For Shigellosis.		
Study Period: Each subject will be followed up for approximately 1 month after last vaccination. Following the screening period, the total study duration will be approximately 2 months for each subject.		Clinical Phase: Phase 2a
Background and Rationale: Shigellosis remains a major health problem in developing countries with approximately 100 million cases per year mostly in children ≤ 5 years. Antibiotic resistance of <i>Shigella</i> is increasing and no vaccine is currently available against shigellosis. Among the <i>Shigella</i> serotypes that are epidemiologically more relevant, the investigators of the recently published GEMS study, aimed to identify the main causes of moderate to severe diarrhea (MSD) in Africa and Asia (Livio et al., 2014) have concluded that a quadrivalent vaccine containing <i>S. sonnei</i> and 3 serotype/subserotypes of <i>S. flexneri</i> (<i>S. flexneri</i> 2a, <i>S. flexneri</i> 3a, and <i>S. flexneri</i> 6) can provide broad coverage against <i>Shigella</i> serotypes (up to 65%), which cause shigellosis in the developing world, and can also provide broad coverage for travelers. The GVGH candidate vaccine against shigellosis caused by <i>Shigella sonnei</i> (1790GAHB) has been developed with a novel technology based on high yield production of Generalized Modules for Membrane Antigens (GMMA) from <i>S. sonnei</i> . GMMA are outer membrane particles naturally released from the <i>S. sonnei</i> during growth.		

Name of Sponsor: GSK Vaccines Institute for Global Health (GVGH)	Protocol number: H03_04TP	Generic name of study vaccine(s): <i>S. sonnei</i> 1790GAHB Vaccine
<p>The candidate <i>S. sonnei</i> 1790GAHB vaccine was chosen as a prototype Shigella vaccine based on the GMMA technology since <i>S. sonnei</i> is among the most common serotypes causing dysentery in humans.</p> <p>This GVGH <i>S. sonnei</i> 1790GAHB vaccine has been tested in two parallel Phase 1 trials in European adult population: H03_01TP looking at the intramuscular (IM) administration and H03_02TP looking at the intradermal (ID), intranasal (IN) and IM administration. Based on the preliminary results from these phase 1 studies, it has been decided to proceed with further development using only the IM route of immunization and to test in the proposed H03_04TP study two doses selected based on the results from H03_01TP study.</p> <p>The two doses have been selected as follows: 25 µg is the lowest dose that in phase 1 consistently induced antibody titers comparable to antibodies in a population of convalescent subjects after natural infection while 100 µg is tested for two reasons i.e.1) a higher dosage may be needed to induce high antibody titers in endemic population and 2) there is a need to test in African populations the tolerability of a higher dose of GMMA that will be ultimately needed in the multivalent vaccine against Shigellosis.</p> <p>The purpose of H03_04TP study is to evaluate the safety and the immunogenicity of two different doses (25 µg and 100 µg) of the GVGH <i>S. sonnei</i> 1790GAHB vaccine in healthy adults and represents the first step towards testing of the GMMA vaccine in the vaccine target population of children from developing countries where shigellosis is endemic.</p>		
<p>Study Objectives:</p> <p>Primary Objective:</p> <p>To evaluate the safety profile of two injections of <i>S. sonnei</i> 1790GAHB vaccine in healthy adults in a <i>Shigella</i> endemic country</p> <p>Secondary Objective:</p> <p>To evaluate the immunogenicity profile of two injections of <i>S. sonnei</i> 1790GAHB</p>		

Name of Sponsor: GSK Vaccines Institute for Global Health (GVGH)	Protocol number: H03_04TP	Generic name of study vaccine(s): <i>S. sonnei</i> 1790GAHB Vaccine										
vaccine in healthy adults by measuring the anti-LPS <i>S. sonnei</i> serum IgG.												
Study Design: This is a phase 2a, randomized, controlled, single center, observer blind trial that will enroll approximately 72 healthy adult subjects, 18-45 years of age inclusive. As currently there is no vaccine available against shigellosis, the safety of the 1790GAHB vaccine will be evaluated against two licensed control vaccines: one dose of Menveo as 1 st injection and one dose of Boostrix as 2 nd injection. Subjects will be randomized to one of the three parallel treatment arms in a 1:1:1 ratio to receive either the study vaccine dose A (25 µg), the study vaccine dose B (100 µg) or the control vaccines as follows: Table 1 Overview of the Study Groups <table border="1"><thead><tr><th></th><th>No. Subjects</th></tr></thead><tbody><tr><td>Group A (25 µg)</td><td>24</td></tr><tr><td>Group B (100 µg)</td><td>24</td></tr><tr><td>Group C (Control)</td><td>24</td></tr><tr><td>Total</td><td>72</td></tr></tbody></table> Two injections of the study vaccine or the two control vaccines will be administered 28 days apart. The study includes a screening visit (performed at study Days -28/-1), five Clinical Visits (performed at study Day 1, 7, and 28 days after 1 st injection and 7 and 28 days after 2 nd injection) and daily home visits performed for 6 days after the 1 st and 2 nd injection. During the screening period before 1 st vaccination, subjects providing informed consent will be screened for general health status. Blood (approximately 10 mL) will be drawn from all subjects before the first injection (visit 1), 28 days after the first injection (visit 3), and 28 days after the second injection				No. Subjects	Group A (25 µg)	24	Group B (100 µg)	24	Group C (Control)	24	Total	72
	No. Subjects											
Group A (25 µg)	24											
Group B (100 µg)	24											
Group C (Control)	24											
Total	72											

Name of Sponsor: GSK Vaccines Institute for Global Health (GVGH)	Protocol number: H03_04TP	Generic name of study vaccine(s): <i>S. sonnei</i> 1790GAHB Vaccine
<p>(visit 5) for immunogenicity evaluation.</p> <p>Appropriately trained study staff will be instructed to complete the subject's diary card during daily home visits following discussion with the subject to (i) describe solicited local (i.e. injection site erythema, induration and pain) and systemic (i.e. fever [temperature $\geq 38.0^{\circ}\text{C}$ measured axillary], fatigue, malaise, myalgia, chills, arthralgia and headache) adverse events (AEs) occurring during the day of each injection visit (visit 1 and 3) and for the following 6 days; (ii) indicate if any analgesic/antipyretic to prevent or treat pain/fever was taken after injection.</p> <p>In addition to the solicited adverse events data, from study visit 1 through visit 5 (i.e. study termination visit) any unsolicited AE, all serious adverse events (SAEs), all AEs leading to vaccine/study withdrawal, all Adverse Events of Special Interest (AESI, see below) and all concomitant medications associated with those events, will be collected and recorded in the subject's source document and on an Adverse Events CRF(s). In case of such events, subjects will be instructed to contact the site as soon as possible to report the event(s).</p> <p>Reactive arthritis or neutropenia will be collected and analyzed as AESIs for this study.</p>		
Number of Subjects planned: A total of 24 subjects per group will be enrolled in each of the three study groups in order to have 20 evaluable subjects in each group. This allows for a drop-out rate of 15% during the course of the trial. Thus a total of approximately 72 subjects will be enrolled in the trial.		
Study Population and Subject Characteristics: Subjects, who meet all inclusion criteria and none of the exclusion criteria (refer to section 4. Selection Of Study Population), will be eligible for enrollment.		
Study Procedures: Before any study procedure is performed, informed consent will be obtained and subjects will be enrolled only after their eligibility for participation is confirmed by the		

Name of Sponsor:	Protocol number:	Generic name of study vaccine(s):
GSK Vaccines Institute for Global Health (GVGH)	H03_04TP	<i>S. sonnei</i> 1790GAHB Vaccine
<p>investigator.</p> <p>Study will be explained in either Swahili or in English where prospective participants express a preference. For subjects who are illiterate the whole informed consent document will be explained in the presence of an impartial witness.</p> <p>For all women of childbearing potential the screening will include the evaluation of human chorionic gonadotropin (hCG) in blood to exclude pregnancy and women refusing to perform the test or with positive test will be immediately excluded from the study. Informed consent process will include counseling about pregnancy and family planning. hCG in urine will then be evaluated (using a dipstick) at visit 1 before randomization; it will be repeated on visit 3 before 2nd vaccination and on the day of the last study visit (visit 5). The pregnancy test should be negative prior to each injection. All pregnancies that occur during the study will be recorded into the Pregnancy CRF appropriate pages and followed until delivery or abortion, as applicable.</p> <p>Female subjects of child-bearing potential must use acceptable birth control measures (defined as oral, injected or implantable contraceptives) for the two months preceding 1st vaccination and during entire study participation.</p> <p><u>Vaccination Procedure:</u> Subjects will receive the study vaccines at study Day 1 (visit 1) and at study Day 29 (visit 3) in accordance with group assignment in a blinded fashion. Vaccines should be administered by unblinded study personnel by deep IM injection into the deltoid area of the non-dominant arm unless there is a reason to vaccinate elsewhere which should be documented. After receiving the vaccination, subjects will be observed for at least 1 hour for any immediate reactions.</p> <p><u>Procedure for collection of solicited AE:</u> appropriately trained study staff will record solicited local and systemic adverse events and medications/vaccinations taken/received by the subject into a Diary Card and following discussion with the subject. Beginning in the evening following study vaccine administration (approximately 6 hours), and daily thereafter through the following 6 days, solicited local and systemic adverse events including other reactions (i.e. body temperature measurements and use of analgesics/antipyretics) will be reported daily by field workers on a Diary Card. For this purpose daily home visits will be performed for 6 days after each injection and a consultation at the vaccination clinic will be performed at 7 days after each injection</p>		

Name of Sponsor:	Protocol number:	Generic name of study vaccine(s):
GSK Vaccines Institute for Global Health (GVGH)	H03_04TP	<i>S. sonnei</i> 1790GAHB Vaccine
<p>(clinic Visits 2 and 4).</p> <p>Any unsolicited AE occurring during the day of injection with study vaccines and for the following 28 days, solicited local and systemic AE that continue beyond 7 days after study vaccination, all SAEs, AEs leading to vaccine/ study withdrawal, AESI and concomitant medications associated with those events will be collected and recorded from Visit 1 to Visit 5 in the subject's records and on the Adverse Events CRF. The information obtained by study personnel will be recorded on the appropriate web based system, referred to as Electronic Data Capture (EDC).</p> <p><u>Safety procedures:</u> All subjects providing informed consent will undergo a general physical examination at the screening visit (study Day -28/-1) and at visit 1 (Day 1) for evaluation of the general health status by clinical assessment, verification of inclusion and exclusion criteria and review of medical history. On visit 3, prior to 2nd injection with the investigational vaccine or control, subjects will be assessed for continued eligibility with regard to the inclusion and exclusion criteria and physical examination. A brief symptom-directed physical examination (if necessary according to symptoms the subject has reported) will be performed at Clinic visits 2, 4 and 5.</p> <p><u>Blood Draw Procedure:</u> Approximately 10 mL of blood will be obtained as part of the initial screening for hematology, renal, and liver function tests, and serological tests for hepatitis B and HIV. In addition, an analysis of the urine will be performed. Each randomized subject will have 10 mL of blood drawn before the 1st and 2nd vaccination, and 28 days after the 2nd vaccination for immunological studies. Additional blood draws of 10 mL for hematology, renal and liver panels will be obtained 7 and 28 days after 1st and 2nd vaccination, urinalysis will be conducted at the same time points.</p> <p>All subjects will undergo study termination procedures on visit 5 (28 days after 2nd injection visit).</p>		
<p>Study Vaccines:</p> <p>GVGH <i>S. sonnei</i> (1790GAHB) vaccine</p> <p>The investigational agent is the GVGH <i>S. sonnei</i> vaccine. The vaccine consists of <i>S. sonnei</i> 1790-GMMA (approximately 200 µg/mL, measured by protein content)</p>		

Name of Sponsor:	Protocol number:	Generic name of study vaccine(s):
GSK Vaccines Institute for Global Health (GVGH)	H03_04TP	<i>S. sonnei</i> 1790GAHB Vaccine
<p>adsorbed to Alhydrogel (0.7 mg Al³⁺/mL) in Tris-buffered saline. The vaccine does not contain any preservative and is available as a liquid formulation in single dose vials with 0.7 mL of injectable solution containing approximately 140 µg of GMMA (as protein content), adsorbed onto 0.49 mg Al³⁺.</p> <p>The vaccine will be used at two different antigen doses obtained by bed-side mixing. Following dilution with Alhydrogel (0.7 mg Al³⁺/mL) in Tris-buffered saline, the volume administered will be 0.5 mL for both doses:</p> <p>Group A: Each 0.5 mL dose of 1790GAHB will contain approximately 25 µg of GMMA total protein and 0.35 mg of Al³⁺.</p> <p>Group B: Each 0.5 mL dose of 1790GAHB will contain approximately 100 µg of GMMA total protein and 0.35 mg of Al³⁺.</p> <p>Bedside mixing, possibly necessary additional labelling of clinical study materials at the study site and vaccine administration will be performed according to GVGH instructions and by trained site staff. The site staff responsible for these activities will be personnel who are respectively qualified according to applicable laws and regulations. GVGH will provide specific procedures and training for these activities.</p> <p>Control vaccines</p> <p>Menveo: vaccine indicated for active immunization to prevent invasive meningococcal disease caused by <i>Neisseria meningitidis</i> serogroups A, C, Y and W-135. The vaccine contains no preservative or adjuvant. Each 0.5 mL dose of vaccine contains 10 µg MenA oligosaccharide, 5 µg of each of MenC, MenY and MenW-135 oligosaccharides conjugated to 32.7 to 64.1 µg of CRM₁₉₇ protein.</p> <p>Boostrix: Vaccine indicated for active booster immunization against tetanus, diphtheria, and pertussis. It contains tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis antigens (inactivated pertussis toxin [PT], formaldehyde-treated filamentous hemagglutinin [FHA] and pertactin). Each antigen is individually adsorbed onto aluminum hydroxide.</p>		

Name of Sponsor: GSK Vaccines Institute for Global Health (GVGH)	Protocol number: H03_04TP	Generic name of study vaccine(s): <i>S. sonnei</i> 1790GAHB Vaccine
No other concomitant vaccines or treatments will be used as part of study procedures.		
Primary Endpoint(s): The safety profile of the two injections of the investigational vaccine and control(s) will be assessed through measurement of the following: <ul style="list-style-type: none">a. Number and percentage of subjects with solicited local and systemic adverse reactions during 7 days following each vaccination.b. Numbers and percentage of subjects with deviations from normal ranges of safety laboratory data after each vaccination.c. Number and percentage of subjects with reported unsolicited adverse events during 28 days following each vaccination.d. Number and percentage of subjects with reported SAEs throughout the study duration.e. Number and percentage of subjects with reported reactive arthritis or neutropenia (defined AESIs).		
Secondary Endpoint(s): The measures of the immunogenicity outcome, (i.e., the anti-LPS <i>S. sonnei</i> serum IgG), will include: <ul style="list-style-type: none">a. IgG geometric mean concentrations (GMCs) pre-vaccination (Day 1), 28 days after 1st vaccination and 28 days after 2nd vaccination, as determined by Enzyme-linked Immunosorbent Assay (ELISA), and applicable geometric mean ratios between post- and pre-vaccination samples.b. Number and percentage of subjects with seroresponse for anti- LPS <i>S. sonnei</i> at 28 days after 1st vaccination and 28 days after 2nd vaccination Seroresponse is aimed to define a significant increase in post vaccination samples based on the biological performance of this specific serology assay and it is defined as: <ul style="list-style-type: none">- If the baseline value is greater than 50 ELISA Units (EU) then an increase of at		

Name of Sponsor:	Protocol number:	Generic name of study vaccine(s):
GSK Vaccines Institute for Global Health (GVGH)	H03_04TP	<i>S. sonnei</i> 1790GAHB Vaccine
<p>least 50% in the post-vaccination sample as compared to baseline [i.e. ((Post-vac minus baseline)/baseline)100% \geq 50%]</p> <ul style="list-style-type: none"> - If the baseline value is less or equal to 50 EU then an increase of at least 25 EU in the post-vaccination sample as compared to baseline [i.e. (post-vac minus baseline) \geq 25 EU] <p>c. Number and percentage of subjects with titers post vaccination concentration \geq 121 for anti-LPS <i>S. sonnei</i> at 28 days after 1st vaccination and 28 days after 2nd vaccination</p> <p>d. IgG GMCs pre-vaccination (Day 1), 28 days after 1st vaccination and 28 days after 2nd vaccination by antibody titer at baseline (i.e., above vs. below the assay detection limit), as determined by ELISA, and applicable geometric mean ratios between post- and pre-vaccination samples.</p> <p>e. Number and percentage of subjects with seroresponse for anti- LPS <i>S. sonnei</i> at 28 days after 1st vaccination and 28 days after 2nd vaccination by antibody titer at baseline (i.e., above vs. below the assay detection limit).</p> <p>A post vaccination concentration \geq 121 anti-LPS serum IgG units in the GVGH ELISA corresponds to a titer of 1:800 in the ELISA method used by Cohen et al. (1989 J. Clin. Microbiol. 27:162). This antibody level is the median antibody concentration of a set of 87 convalescent subjects previously infected by <i>S. sonnei</i>. The value of 121 anti-LPS serum IgG units in the GVGH ELISA was determined by calibration against the Cohen ELISA (i.e., the GVGH standard serum was tested in Cohen's lab using the Cohen's methodology).</p> <p>Exploratory endpoints</p> <p>Other assays, including serum secretory IgA, might be done to further characterize the immune response to the study vaccine.</p>		
<p>Statistical Analyses:</p> <p>This Phase 2a safety and immunogenicity study is aimed to descriptively evaluate the safety and immunogenicity profiles of the study vaccines. No specific hypotheses are tested in this trial. A total of 72 subjects will be enrolled in this study: 24 subjects per</p>		

Name of Sponsor: GSK Vaccines Institute for Global Health (GVGH)	Protocol number: H03_04TP	Generic name of study vaccine(s): <i>S. sonnei</i> 1790GAHB Vaccine
group will be enrolled in order to have 20 evaluable subjects in each group (15% drop-out rate). With 20 evaluable subjects in each vaccine group, the probability of observing at least one adverse event per group is 87%, if the actual rate of the event is 10%.		
Interim Analysis: For up to 45 subjects, once they have completed the study, a group unblinded preliminary immunogenicity analysis and a blinded interim safety analysis will be performed. Individual subject results from preliminary analyses will not be made available to site and sponsor personnel until the end of the study. The results of these immunogenicity and safety analyses will not impact the conduct of the study, but will inform the planning of future studies with the same vaccine (see also section 8.6 of the protocol).		
Data Monitoring Committee: An independent data safety monitoring board (DSMB) will receive a summary of all safety data (solicited local and systemic AE, unsolicited AE and SAEs) and listings of clinically significant modifications in hematology, blood chemistry and urine dipstick/urinalysis test values obtained during one week follow-up post-first vaccination of the initial 18 subjects. Further recruitment will be put on hold until the DSMB opinion is received. Throughout the entire study the DSMB will be consulted for any safety issue that might be reported during the trial. See section 3.8 of the protocol for more details.		

Table 2 Time and Events Table

Study Periods		Screening	Treatment						
Visit Type		Clinic	Clinic	Home visits	Clinic	Clinic	Home visits	Clinic	Clinic
Visit Number		n/a	1	Daily for 6 days	2	3	Daily for 6 days	4	5
Study Day*		n/a	1		V1+7	V1+28		V3+7	V3+28
Time Window (days) (min/max)		-28/-1	0	0	0/+1	0/+7	0	0/+1	0/+7
Study Event	References								
Study Treatment									
Vaccination	Section 5.2		X			X			
Screening and Safety									
Informed Consent ^[a]	Section 5.1.1	X							
Medical History	Section 5.1.2	X							
Physical Examination ^[b]	Sections 5.1.2 and 5.3.1	X	X		X	X		X	X
Pregnancy Test	Sections 5.5.2 and 5.1.2	X (blood)	X (urine)			X (urine)			X (urine)
Inclusion/Exclusion Criteria ^[c]	Section 4.0	X	X			X			
Randomization	Section 5.1.4		X						
Blood draw for Serology for HIV, HBV	Section 5.1.2	X							
Safety blood draw ^[d]	Sections 3.5 and 5.1.2	X			X	X		X	X
Urine dipstick (urinalysis as required)	Sections 3.5 and 5.1.2	X			X	X		X	X
Post Injection Assessment ^[e]	Sections 5.1.2		X			X			

Study Periods		Screening	Treatment						
Visit Type		Clinic	Clinic	Home visits	Clinic	Clinic	Home visits	Clinic	Clinic
Visit Number		n/a	1	Daily for 6 days	2	3	Daily for 6 days	4	5
Study Day*		n/a	1		V1+7	V1+28		V3+7	V3+28
Time Window (days) (min/max)		-28/-1	0	0	0/+1	0/+7	0	0/+1	0/+7
Study Event	References								
Post Injection Assessment ^[f]	Sections 5.2.1 and 5.3.1 and 5.3.2		X	X	X	X	X	X	
Assess all AEs ^[g]	Section 7.1		X	X	X	X	X	X	X
Assess/ Inquire about AE leading to withdrawal, all SAE and AESI ^[h]	Sections 7.1.4.1 and 7.1.3		X	X	X	X	X	X	X
Assess Medications and Vaccinations	Sections 5.1.2 and 6.5	X	X	X	X	X	X	X	X
Study Termination	Section 5.5								X
Immunogenicity									
Serology blood draw	Sections 3.5 and 7.3		X			X			X

* Study days should be calculated based on the actual date of the previous visit (as to comply with requested Study Visit Time Window)

- Informed Consent to be obtained before any study procedure
- Physical examination must be performed by a qualified health professional in accordance with local regulations and licensing requirements designated within the Site Responsibility Delegation Log.
- Compliance with Exclusion/Inclusion criteria should be verified.
- In case of neutropenia, Complete Blood Count to be repeated on a weekly basis until resolution. If neutropenia occurs at the last study visit, Complete Blood Count to be repeated on a regular basis until resolution.
- A post-injection local and system adverse event and body temperature measurement will be performed approximately 30 and 60 minutes after each vaccination during the clinic visit.

- f. Beginning in the evening following study vaccine administration (approximately 6 hours), and daily thereafter through the following 6 days, solicited local and systemic adverse events including body temperature measurements and use of analgesics/antipyretics will be reported by field workers in a diary card based on subject's observation and interview.
- g. All unsolicited adverse events will be captured through 28 days following each vaccination.
- h. SAEs, AESI and AEs leading to study or vaccine withdrawal will be collected through entire study duration.

Table 3 Safety Tests Table

HEMATOLOGY
White Blood Cells (WBC)
Red Blood Cells (RBC)
Haemoglobin
Haematocrit
Platelets
Eosinophils
Basophils
Neutrophils
Monocytes
Lymphocytes
Prothrombin time*
CLINICAL CHEMISTRY
Total bilirubin
Aspartic Aminotransferase (ASAT/GOT)
Alanine Aminotransferase (ALAT/GPT)
γ -Glutamyl Transferase (γ -GT)
Lactic Dehydrogenase (LDH)
Alkaline Phosphatase (AP)
Total Proteins*
Glucose (random glucose)
Blood Urea Nitrogen (BUN)
Creatinine
Sodium
Potassium
SEROLOGY for VIROLOGY
HbsAg*
HIV antibodies*
PREGNANCY TEST
Human chorionic gonadotropin (hCG) in blood *
Human chorionic gonadotropin (hCG) in urine

* Performed at screening only

URINE DIPSTICK	
Glucose	
Proteins	
pH	
Ketones	
Nitrites	
Blood	
URINALYSIS: Microscopic test on urine (performed if urine dipstick shows deviations from normal values)	
Leucocytes (WBC)	
Erythrocytes (RBC)	
Epithelial Cells	
Casts	
Bacteria	

LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Events of Special Interest
AP	(Statistical) Analysis Plan
ANC	Absolute Neutrophil Count
BMI	Body mass index
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CI	Confidence Interval
CRF	Case Report Form
CRO	Contract Research Organization
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EDT	Electronic Data Transfer
ELISA	Enzyme-linked Immunosorbent Assay
EMA	European Medicines Agency
FAS	Full Analysis Set
FDA	Food and Drug Administration
GCP	Good Clinical Practices
GMC	Geometric Mean Concentration
GMMA	Generalized Modules for Membrane Antigens
GMR	Geometric Mean Ratio
GMT	Geometric Mean Titer
HIV	Human Immunodeficiency Virus
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ID	Intradermal
IM	Intramuscular
IN	Intranasal
IV	Intravenous
IRB	Institutional Review Board
ITT	Intention-To-Treat
MITT	Modified Intention-To-Treat
MSD	Moderate to Severe Diarrhea
LPS	Lipopolysaccharide
LSLV	Last Subject Last Visit

MedDRA	Medical Dictionary for Regulatory Activities
NOCD	New onsets of chronic diseases
OAg	O antigen (of <i>Shigella sonnei</i>)
OMV	Outer Membrane Vesicles
PO	Per Os (orally)
PP	Per Protocol
PSB	Product Stewardship Board
ReA	Reactive arthritis
SAE	Serious Adverse Event
GVGH	GSK Vaccines Institute for Global Health
SDA	Source Data Agreement
SOC	System Organ Class
SOP	Standard Operating Procedure
SPC	Summary of Product Characteristics
WHO	World Health Organization

1. BACKGROUND AND RATIONALE

Shigella spp. are Gram-negative bacteria that infect the intestinal epithelium and are major causes of diarrhea, including dysentery. *Shigella* is transmitted by the fecal-oral route and taken up by contaminated food or water. It is endemic throughout the world but the main burden of disease is in developing countries. In 2009, the World Health Organization (WHO) estimated approximately 125 million cases of shigellosis per year in Asia alone (Bardhan et al., 2010). Ninety-nine percent of all cases occur in developing countries and approximately 70% in children younger than 5 years of age (Kotloff et al., 1999). Current estimates of mortality vary between 108,000 worldwide (http://www.who.int/vaccine_research/diseases/diarrhoeal/en/index6.html) and 14,000 in Asia where previously 80% of all deaths were estimated to occur. Sixteen serotypes (all 14 *S. flexneri*, *S. sonnei*, and *S. dysenteriae* type I) are considered to be of global importance (Zhang et al., 2011) with *Shigella sonnei* being the most common serotype worldwide.

Among the *Shigella* serotypes that are epidemiologically more relevant, the investigators of the recently published GEMS study, aimed to identify the main causes of moderate to severe diarrhea (MSD) in Africa and Asia (Livio et al., 2014) have shown that 8 *Shigella* serotypes are approximately responsible for 87% of the disease. However, they concluded that a quadrivalent vaccine containing *S. sonnei* and 3 serotype/subserotypes of *S. flexneri* (*S. flexneri* 2a, *S. flexneri* 3a, and *S. flexneri* 6) can provide a sufficiently broad coverage against *Shigella* serotypes (up to 65%), which cause the majority of endemic pediatric shigellosis in the developing world, and can also provide broad coverage for travelers.

In target populations, treatment options for shigellosis are limited. Shigellosis can be treated with appropriate antibiotics. However, antibiotic resistance is increasing and many *Shigella* isolates are resistant to two or more of the common antibiotics ampicillin, chloramphenicol, nalidixic acid, co-trimoxazole. Resistance to third generation antibiotics, especially ciprofloxacin, has been reported to be emerging (Zhang et al., 2011). Still effective antibiotics include ceftriaxone that is administered intramuscularly or intravenously and is not easily accessible for people in impoverished communities.

No vaccine is available. Natural infection (experimental infection or vaccination with attenuated *Shigella*), leads to good protective immunity, but despite the generally high genetic conservation between serotypes, the protection is highly specific for the infecting serotype. This suggests that the dominant protective antigen is the O antigen (OAg) of the lipopolysaccharide (LPS). There have been many attempts to make a *Shigella* vaccine, using inactivated whole cell bacteria either orally or parentally (low efficacy, high reactogenicity for parenteral), attenuated live oral vaccines (no vaccine has yet obtained a useful balance between attenuation and efficacy), recombinant surface proteins (several projects at an early stage), O antigen conjugates (Levine et al., 2007). The latter have been the most successful to date with a parenteral *S. sonnei* and *S. flexneri* type 2OAg

conjugates tested in field trials in Israel that achieved 74% efficacy in adults (Cohen et al., 1997) with one immunization and 71% efficacy in children 3 years of age and older with 2 vaccinations (Passwell et al., 2003, Passwell et al., 2010). No significant efficacy was achieved in younger children in accordance with a very low immunogenicity in the young children. As expected for an OAg vaccine, this was highly serotype specific and the *S. sonnei* OAg afforded no protection against infection with *S. flexneri* nor did a *S. flexneri* 2a based OAg conjugate protect against *S. sonnei*. In addition, to achieve broad-spectrum protection against the 16 serotypes that are currently considered to be globally important, a multivalent OAg-vaccine will be needed. Therefore, new vaccine development approaches are needed.

GSK Vaccines Institute for Global Health (GVGH) candidate vaccine against shigellosis caused by *Shigella sonnei* (1790GAHB) is based on a parenteral vaccine targeting the OAg but using a new platform technology called Generalized Modules for Membrane Antigens (GMMA) as a novel delivery system which may be applicable also for other vaccines against Gram-negative pathogens. GMMA are naturally shed from the surface of Gram-negative bacteria and consist of outer membrane proteins, outer membrane lipids, including phospholipids and LPS, and enclosed periplasmic proteins (Beveridge, 1999). In the previous literature, GMMA are called outer membrane vesicles (OMV). However, this term has also been used for vesicles derived by detergent-extraction of homogenized bacteria which are used as vaccines, e.g. to control *Neisseria meningitidis* type B infections in New Zealand (MeNZB) or other broader coverage MenB vaccines (Bexsero). In order to clearly differentiate these different types of particles, the name 'GMMA' was introduced for the blebs spontaneously released from the cell surface (Berlanda Scorza et al., 2012). The natural arrangement of the outer membrane is preserved during the release of GMMA and therefore GMMA allow an optimal exposure of the antigens of the outer membrane for recognition by the host immune system. GVGH has developed an economic process to purify GMMA in large quantities from high density cultures of bacteria genetically modified to increase GMMA production and generate a LPS with low endotoxicity, suitable for use in humans. *S. sonnei* was chosen as the proof of concept for the GMMA technology and as a prototype *Shigella* GMMA vaccine since *S. sonnei* is among the most common serotypes causing dysentery in humans.

A comprehensive review of GVGH 1790GAHB vaccine is contained in the Investigator's Brochure (IB) supplied by GVGH; this document should be reviewed prior to initiating the study.

1.1 Background

This GVGH *S. sonnei* 1790GAHB vaccine has been tested in two Phase 1 trials in European adult population: H03_01TP, looking at the intramuscular (IM) administration,

and H03_02TP, looking at the intradermal (ID), intranasal (IN) and IM administration. Based on the results from these phase 1 studies, it has been decided to proceed with further development using only the IM route of immunization and to test in the proposed H03_04TP study two doses selected based on the results from H03_01TP study. The two doses have been selected as follows: 25 µg is the lowest dose that in phase 1 induced antibody titers comparable to antibodies in a population of convalescent subjects after natural infection already after the first vaccination, while 100 µg is tested for two reasons i.e. 1) a higher dose may be needed to induce high antibody titers in endemic population and 2) there is a need to test in African populations the tolerability of a higher dose of GMMA that will be ultimately needed in the multivalent vaccine against Shigellosis.

The proposed H03_04TP trial is the logical continuation of the two Phase 1 studies and is aimed to evaluate the safety and immunogenicity profile of 1790GAHB in adults from an African country, where shigellosis remains a major health problem.

The dose regimen tested in phase 1 studies (three doses given 1 month apart) did not show any significant benefit from the third dose in terms of immunogenicity, therefore a two dose schedule was selected for the current trial. The two dose schedule will allow evaluation of the immunogenicity in the endemic population where one dose may be not enough to induce the same immunogenicity observed in European population.

During the two phase 1 trials two subjects of African descent experienced a transient and clinically asymptomatic decrease of circulating neutrophils of grade 3 (severe) that was finally classified as "benign ethnic neutropenia". In addition, six more subjects experienced transient and clinically asymptomatic grade 2 (moderate) neutropenia. Although this finding was not associated with any clinical illness, some precautionary procedures for enrollment and monitoring of trial subjects have been introduced to protect the safety of study subjects (refer to [Section 3.7, Procedures Related to Neutropenia](#)).

The trial will be conducted in compliance with the protocol, GCP and applicable regulatory requirement(s).

1.2 Rationale

The purpose of H03_04TP study is to evaluate the safety and the immunogenicity of two different doses (25 µg and 100 µg) of the GVGH *S. sonnei* 1790GAHB vaccine in healthy adults in Africa and represents the first step towards testing of the GMMA vaccine in the vaccine target population of children from developing countries where Shigellosis is endemic.

2. OBJECTIVES

2.1 Primary Objective(s)

Primary Safety Objective(s)

To evaluate the safety profile of two injections of *S. sonnei* 1790GAHB vaccine in healthy adults in a Shigella endemic country

2.2 Secondary Objective(s)

Secondary Immunogenicity Objective(s)

To evaluate the immunogenicity profile of two injections of *S. sonnei* 1790GAHB vaccine in healthy adults at 28 days after 1st vaccination and 28 days after 2nd vaccination, by measuring the anti-LPS *S. sonnei* serum IgG.

2.3 Exploratory Objective(s)

This study has no exploratory objectives.

3. STUDY DESIGN

3.1 Overview of Study Design

This is a phase 2a, randomized, observer blind, controlled single center study in healthy adult women and men healthy volunteers evaluating the safety and immunogenicity of two doses of the GVGH vaccine against *Shigella sonnei* infections (1790GAHB). As no vaccine is currently available against shigellosis, the safety profile of the 1790GAHB vaccine will be evaluated in comparison to that of two control vaccines, Menveo as 1st vaccination and Boostrix as 2nd vaccination. All subjects and blinded study personnel will be blinded to treatment throughout the study.

This clinical trial has been designed to minimize pain, discomfort, fear and any other foreseeable risks. During the screening period, subjects giving informed consent will be screened for general health status. No pharmacokinetic tests will be performed as evaluation of pharmacokinetic properties is not required for vaccines unless new delivery systems are employed or when the vaccine contains novel adjuvants or excipients (Berlanda Scorza et al., 2012). Subjects who meet all inclusion criteria and none of the exclusion criteria, with screening tests within normal values and women of child bearing potential with negative pregnancy test will be eligible for enrollment. Female subjects of child bearing potential must use birth control measures during study participation.

A total of 72 eligible subjects will be assigned to one of the three parallel treatment arms in a 1:1:1 ratio to receive either the study vaccine dose A (25 µg), the study vaccine dose B (100 µg) or the control vaccines as follows:

Table 3.1-1 Overview of the Study Groups

	No. Subjects
Group A (25 µg)	24
Group B (100 µg)	24
Group C (Control)	24
Total	72

Subjects will be randomized to one of the three vaccine groups and all subjects will receive two intramuscular vaccinations, four weeks apart.

Screening/baseline clinical safety labs will take place before Visit 1 (from -28 up to -1 day). Blood will be obtained as part of the initial screening for hematology, renal, and liver function tests, and serological tests for hepatitis B and HIV. In addition, an analysis of the urine will be performed. Each randomized subject will have blood collected before the 1st and 2nd vaccination, and 28 days after the 2nd vaccination for immunological

testing. Additional blood draws for hematology, renal and liver panels will be obtained 7 and 28 days after 1st and 2nd vaccination, urine dipstick/urinalysis will be conducted at the same time points. All individuals with a neutropenia occurring at any time during the study will have additional blood draws to repeat complete blood count on a weekly basis until the neutropenia resolves (for classification of neutropenia during the trial, refer to [Section 3.7, Procedures Related to Neutropenia](#)).

Subjects will be observed at the clinic for at least 1 hour after each vaccination. In order to avoid excluding participants who cannot read and write, safety information (solicited local and systemic AE including body temperature measurements and use of analgesics/antipyretics) and medications/ vaccinations received beginning in the evening following study vaccine administration and daily thereafter through the following 6 days, will be recorded by appropriately trained study staff in a Diary Card, following discussion with the subject. For this purpose daily home visits will be performed on the day of the injection and for 6 days after each injection and a consultation at the vaccination clinic will be performed at 7 days after each injection (clinic Visits 2 and 4).

Any unsolicited AE, serious adverse events (SAEs), all AEs leading to vaccine/study withdrawal, reactive arthritis and neutropenia (adverse event of special interest (AESI)) and all concomitant medications associated with those events, will be collected for the entire study and recorded in the subject's source document.

3.2 Study Period

Each subject should expect to participate in the study for 2 months, from the time of enrolment through the last study visit.

3.3 Blinding Procedures

The identity of the study vaccine and control cannot be concealed as presentations are different.

Therefore an observer blind study design has been chosen: during the study, designated unblinded trained and qualified site staff (please see [section 5.3](#)) will be responsible for preparing the study vaccine or controls out of view of the subject and an unblinded nurse(s) or clinician(s) will be responsible for administering the study vaccines to the subjects. The unblinded staff will be instructed not to reveal the identity of the study vaccines either to the subject or the other investigative site personnel involved in the conduct of the trial. The designated unblinded pharmacists, nurse(s) or clinician(s) will not take part in evaluating the subject(s) for safety or collect study data after the administration of the study vaccine.

Study vaccines allocations will not be available to the investigator or blinded personnel monitoring the trial until after the completion of the trial and final data review. Adherence to the randomization list will be verified by a designated and unblinded Study Monitor, independent of the staff involved in the regular monitoring of the study, by checking the randomization list against the vaccination records maintained at the study site.

Except in the case of medical necessity, a subject's treatment should not be unblinded without the approval of the Sponsor. In such instance of medical emergency, every effort should be made to contact the Sponsor prior to unblinding. If unblinding should occur (by either accidental unblinding or emergency unblinding for a serious adverse event) prior to completion of the study, the investigator must promptly contact the Sponsor and document the circumstances on the appropriate forms. Instructions regarding emergency unblinding will be provided to the investigator.

3.4 Data Collection

3.4.1 Data Collected from Subjects

The following data will be collected from each subject over the duration of their study participation:

- Demographic Information.
- Post-vaccination immediate reactions.
- Body temperature.
- Adverse Events.
- Medical History.
- Concomitant Medications.

All data collected must only be identified using the GVGH Subject ID and Subject code, as described in [section 5.1.4, Randomization](#).

3.4.2 Tools Used for Data Collection

Data will be recorded in a Subject Diary Card by appropriately trained study staff and entered on Case Report Forms (CRFs).

The subject Diary Card will be the only source document allowed for solicited local and systemic adverse events (including body temperature measurements), starting after the initial 60 minute post-vaccination period at the clinic. The following additional rules apply to documentation of safety information collected in the Diary Card.

The Investigator or delegated staff should monitor the Diary Card status throughout the study for compliance and any solicited local and systemic adverse events that were of concern to the subject.

1. No corrections or additions to the information recorded by the trained study staff within the Diary Card will be allowed after it is delivered to the site.
2. Any blank or illegible fields on the Diary Card must be described as missing in the CRF.

Case Report Forms

This study utilizes Case Report Forms (CRFs) to collect study-related data from each subject. A qualified site staff member(s) is required to enter subject data in the CRFs in English based on the medical information available in each subject's source record.

Data should be entered into the CRF in a timely fashion following each subject's clinic visit, study procedure, or home visit. Each subject's CRF casebook will be compared with the subject's source records by a GVGH-approved study monitor (or designee) over the duration of the study in order to ensure data collection accuracy.

3.5 Collection of Clinical Specimens

The following clinical specimens are required to be collected from each subject in this study:

- Blood
- Urine

Blood Specimens

A maximum of 10 mL of blood will be obtained as part of the initial screening. The blood will be used for pregnancy testing in females of child bearing potential and for hematology, renal and liver function tests, and serological tests for hepatitis B and HIV in all subjects as described in [synopsis Table 3, Hematological, Haematochemical Blood Tests and Urinalysis Table](#). The safety laboratory assays will be conducted at the Clinical Trials Laboratory, Kilifi, Kenya.

Each randomized subject will have blood collected before the 1st and 2nd vaccination, and 28 days after the 2nd vaccination for immunological assays. The blood volume will not exceed 10 mL at each time point in order to provide the necessary serum volume (approximately half of the blood draw volume) for the serology assays.

Serum samples will be stored frozen below -20°C. One aliquot of serum will be maintained at the KEMRI-Wellcome Trust laboratory. The other aliquots will be shipped according to guidelines provided by the sponsor to the laboratories for analysis. The serologic assays will be conducted at the GSK Clinical Serology Laboratory, Marburg, (Germany) or a delegated laboratory.

Aliquots of sera will be archived for 15 years both at KEMRI-WT laboratory in Kilifi and GSK laboratory in Marburg (Germany) for future research on immunogenicity of the *Shigella* vaccine. Study-related future research may include additional evaluation of immunogenicity on *Shigella sonnei* (i.e. IgM, IgA against the O antigen or IgG against other antigens of *Shigella sonnei*). Non-study related future research may include serology on other *Shigella* serotypes and other bacteria causing infectious diseases in developing countries (e.g. *Salmonella Typhi*, *Salmonella Paratyphi*, non-typhoidal *Salmonella*, meningitis) to inform development of other vaccines relevant to the populations in developing countries. It may not be possible to contact individual participants in the future in order to disseminate results obtained from research conducted on archived samples. However, these results, if relevant, will be published in open access peer-reviewed journals and disseminated to Kilifi county leaders and the community. An archival time of 15 years should cover the entire vaccine development time, including the possibility to address potential questions from regulators during the registration time.

Complete instructions for processing, labeling, storage and shipping of samples are included in the Clinical Specimen Laboratory Manual provided by sponsor and available in the Investigator Site File.

Additional blood draws of 10 mL for hematology, renal and liver function tests will be obtained 7 and 28 days after 1st and 2nd vaccination.

See [section 7, Assessments](#) for additional details.

The total amount of blood collected over the study period (including the screening) per subject will be 80 mL.

Blood samples must be collected in the appropriate manner, using exclusively materials and guidelines supplied by the sponsor. The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement.

The results of safety testing will be recorded in the source document and CRF.

Urine Specimens

Urine will be collected for urine dipstick/urinalysis as part of the initial screening, before the 2nd vaccination, and 7 and 28 days after the 1st and 2nd vaccination. Urine samples will be also used for pregnancy testing in females of child bearing potential before the 1st and 2nd vaccination, and 28 days after the 2nd vaccination.

The results of urine dipstick/urinalysis will be recorded in the source document and CRF.

3.6 Stopping/Pausing Guidelines

At the end of the 7 days observation period following 1st vaccination of the initial 18 subjects, a summary of all safety data (solicited local and systemic AE, unsolicited AE and SAE), listings of hematology, blood chemistry and urine dipstick/urinalysis test values and respective clinically significant modifications, if any, will be provided to the DSMB. Based on evaluation of the safety data, the DSMB will make a recommendation, as to whether the enrollment can be completed or not. Additionally, DSMB will make a recommendation, as to whether the enrollment of further subjects could be done using the published local normal ranges for neutrophils in Africa (Karita et al., 2009) or should instead be continued using the western normal ranges adopted for the first 18 subjects. Enrollment for the subsequent subjects will be started immediately after DSMB review and confirmation in writing.

During the course of the study, the occurrence of two cases of marked neutropenia (i.e. $< 0.5 \times 10^9/L$), or of febrile neutropenia (i.e., $ANC < 1.0 \times 10^9/L$ associated with fever) will result in study hold, unblinding of data by DSMB (but not by the investigators in the site), discussion of results with safety management team (i.e., Pharmacovigilance) and final decision made in consultation with DSMB, IRB and PI. For additional information, please refer to [Section 3.7, Procedures Related To Neutropenia](#)

Independent of the DSMB, GVGH, as a sponsor, retains the right to halt the study at any time if there is a safety concern. If the study is prematurely terminated, the sponsor will promptly inform Regulatory Authorities and Ethic Committees on the decision of stopping the trial and no further enrollment or study immunizations will occur until written authorization is provided by the sponsor in conjunction with a recommendation to proceed by the DSMB and in consultation with Regulatory Authorities and Ethic Committees, as appropriate.

3.7 Procedures related to neutropenia

During the two phase 1 trials with 1790GAHB, two subjects of African descent experienced a transient and clinically asymptomatic decrease of circulating neutrophils of

grade 3 (severe) that was finally classified as "benign ethnic neutropenia". In addition, six more subjects experienced transient and clinically asymptomatic grade 2 (moderate) neutropenia. Although this finding was not associated with any clinical illness, some precautionary procedures for enrollment and monitoring of trial subjects were introduced to protect the safety of study subjects, including selection of subjects with baseline ANC above $1.8 \times 10^9/L$.

In order to maintain a prudent approach, but, at the same time, to not compromise the scientific validity of the clinical trial by selecting participants that are not entirely representative of the local healthy population (if non-local laboratory reference intervals are used) a step-wise approach will be adopted for the present trial H03_04TP. More specifically, same as in Phase 1 trials, the more conservative western normal ranges will be adopted for ANC collected up to 7 days after 1st vaccination of the initial 18 subjects. .

Subsequently, all safety data from these 18 subjects will be evaluated by the DSMB which will make a recommendation as to whether the enrollment and assessment of neutropenia during the study of further subjects could be done using the local normal ranges published for neutrophils (Karita et al., 2009) and for which a consensus interval of 1.0 to $5.3 \times 10^9/L$ has been reached, or should be continued using the western normal ranges adopted for the first 18 subjects. This is also in consideration of the clinically benign nature of the events reported in the phase 1 studies and of the documented lower absolute neutrophils counts in healthy African populations (one of the key targets for this vaccine) compared to western populations (Badenhorst et al., 1995, Ezeilo, 1972, Lugada et al., 2004),

Therefore, study procedures will be as follows:

- First subset of 18 subjects (prior to DSMB evaluation of data):

Subjects with ANC less than $1.8 \times 10^9/L$ at screening will not be enrolled in the study. All individuals with ANC $< 1.8 \times 10^9/L$, occurring at 7 days after the first dose, will have additional blood draws for complete blood count to be repeated on a weekly basis until the neutropenia resolves (ANC $\geq 1.8 \times 10^9/L$). In case the ANC is less than $0.5 \times 10^9/L$ after 1st vaccination, the subject will be immediately discontinued from 2nd vaccination and will have the test repeated on a weekly basis until the neutropenia resolves.

- All subjects (after DSMB evaluation of safety data from the initial 18 subjects with recommendation to switch to local normal ranges published for neutrophils):

Subjects with neutropenia defined as ANC less than $1.0 \times 10^9/L$ at screening will not be enrolled in the study. All individuals with a neutropenia (ANC $< 1.0 \times 10^9/L$), occurring

at any time during the study 7 days after the first dose, will have additional blood draws for complete blood count to be repeated on a weekly basis until the neutropenia resolves ($ANC \geq 1.0 \times 10^9/L$). In case the ANC is less than $0.5 \times 10^9/L$ after 1st vaccination, the subject will be immediately discontinued from 2nd vaccination and will have the test repeated on a weekly basis until the neutropenia resolves.

For classification and grading of severity of neutropenia for the first subset of 18 subjects, the criteria of Common Terminology Criteria for Adverse Events (CTCAE) issued by US Department of Health and Human Services (version 4.3; 2010) will be adopted. In case DSMB endorses the use of local ranges of normality for the second subset of 54 subjects, the grading will be modified based on input from DSMB. All individuals with neutropenia either ongoing or with onset at visit 5 (or last study visit) will have additional blood draws for complete blood count to be repeated on a regular basis until the neutropenia resolves ($ANC \geq 1.0 \times 10^9/L$ or $ANC \geq 1.8 \times 10^9/L$, depending on recommendations given by DSMB after evaluation of safety data from the initial 18 subjects).

Finally, all cases of neutropenia, regardless of intensity, will be considered as Adverse Events of Special Interest (AESI) from a reporting perspective.

3.8 Data Monitoring Committee

A DSMB will be in place to receive from GVGH a summary of all safety data (solicited local and systemic AEs, unsolicited AEs and SAEs) and listings of clinically significant modifications in hematology, blood chemistry and urine dipstick/ urinalysis test values obtained during one week follow-up post-first vaccination of the initial 18 subjects. Based on evaluation of the safety data, the DSMB will make a recommendation to GVGH, as to whether the enrollment can be completed or not. Additionally, DSMB will make a recommendation to GVGH, as to whether the enrollment of further subjects could be done using the published local normal ranges for neutrophils in Africa (Karita et al., 2009) or should instead be continued using the western normal ranges adopted for the first 18 subjects.

In addition to the evaluation of the safety data from the initial 18 subjects, DSMB will be consulted for any safety issue that might be reported during the trial.

The composition of DSMB and the details of all relevant procedures will be documented in the DSMB Charter.

3.9 Premature Withdrawal from Study

Subjects may withdraw at any time, or be dropped from the study at the discretion of the investigator should any untoward effects occur and/or for safety reasons. In addition, a

subject may be withdrawn by the investigator or the Sponsor if he/she violates the study plan or for administrative reasons. The investigator or study coordinator must notify the Sponsor immediately when a subject has been withdrawn due to an adverse event.

The circumstances above are referred to as premature withdrawal from the study, and the reason for premature withdrawal should be clearly documented and detailed in the source documentation. The investigator should make every attempt to evaluate the subject's safety, including resolution of ongoing AEs, at the time of premature withdrawal. If a subject wants to withdraw from the study before all doses are administered or prior to the last planned study visit, the subject will be asked to be followed for safety for the duration of the study. When a subject withdraws, or is withdrawn, from the study, the procedures described in [section 5.5.1, Early Termination Visit](#) should be completed if possible.

The reasons for premature withdrawal from the study include: Adverse event, death, withdrawal of consent, lost to follow-up, administrative reason, and protocol deviation. These reasons are described in greater detail below.

Adverse Event

For any subject withdrawn from study participation prior to the planned Study Termination Visit, it is important to determine if an AE was associated with the reason for discontinuing the study. This AE must be identified on the AE CRF page by indicating "Withdrawn from study due to AE". Any ongoing AEs at the time of study withdrawal must be followed until resolution or stabilization.

Subjects who develop a serious adverse event (SAE) judged to be possibly or probably related to the study vaccine, including hypersensitivity reactions, should not receive subsequent vaccination.

Death

For any subject withdrawn from study participation due to death, this should be noted on the Study Termination CRF page and the associated SAE that led to the death must be reported.

Withdrawal of consent

The subject can withdraw consent for participation in the study at any time without penalty or loss of benefit to which the subject is otherwise entitled. Reason for early termination should be deemed as "withdrawal of consent" if the subject withdraws from participation due to a non-medical reason (i.e., reason other than AE). If the subject intends to withdraw consent from the study, the investigator should clarify if the subject

will withdraw completely from the study or if the subject will continue study participation for safety, or a subset of other study procedures. If the subject requests complete withdrawal from the study, no further study interventions will be performed with the subject.

Lost to Follow-Up

For subjects who fail to show up for final visits (clinic or home visits), or for three consecutive visits, study staff are encouraged to make at least three documented attempts to contact the subject to encourage the completion of study termination procedures. These efforts to contact the subject should be recorded in the source document. The termination date for the subject to be captured on the Study Termination CRF page is the date of the last successful contact (clinic visit or home visits) with the subject.

Administrative Reason

Examples for subjects withdrawn from the study due to administrative reason can include: Sponsor decision to terminate the study, subject meeting a pre-specified withdrawal criterion, subject discontinuation for insurance issues, moving, no time, etc. This reason should be noted in the Study Termination CRF page and any ongoing AEs at the time of study withdrawal must be followed until resolution/stabilization.

If the clinical study is prematurely terminated by the Sponsor, the investigator is to promptly inform the study subjects and local EC/IRB and should assure appropriate therapy and follow up for the subjects. All procedures and requirements pertaining to the archiving of study documents should be followed. All other study materials (study medication/vaccines, etc.) must be returned to the Sponsor.

For subjects who are withdrawn from the study due to receipt of an excluded medication/vaccination or due to significant protocol non-compliance, this reason should be noted in the Study Termination CRF page.

In studies that involve more than 1 consecutive dose of study vaccine, a separate event is “**withdrawal of study vaccination**”. This event may occur if subjects are expected to receive more than 1 consecutive dose of vaccine as part of study participation. The act of withholding additional study vaccinations is referred to as withdrawal of study vaccination. Subjects may be withdrawn from study vaccination for several reasons including but not limited to: AE related to earlier vaccinations, failure to meet inclusion or exclusion criteria for revaccination (see [section 4.0, Selection Of Study Population](#)), or pregnancy. **Subjects who are withdrawn from study vaccination should be encouraged to continue in the study for safety follow-up and other procedures as appropriate until the scheduled termination visit.** If the subject is withdrawn from

study vaccination(s) due to adverse event, this event must be linked to the withdrawal from vaccination on the AE CRF page

Protocol Deviation

A protocol deviation is any change, divergence, or departure from the study design or procedures of a study protocol. In general, subjects associated with protocol deviations may remain in the study unless continuation in the study jeopardizes the subject's health, safety, or rights.

Investigators will apply due diligence to avoid protocol deviations. Under no circumstances should the investigator contact GVGH or its agents, if any, monitoring the study to request approval of a protocol deviation, as no authorized deviations are permitted. If the investigator feels a change to the protocol would improve the conduct of the study this must be considered a protocol amendment, and unless such an amendment is agreed upon by GVGH and approved by the IRB/EC and health authorities it cannot be implemented.

Any subject who becomes pregnant during the study, despite the protocol requirement for adequate contraception, will not receive further vaccination but should be encouraged to continue participating in the study for safety follow-up. The site must complete a paper Pregnancy Report CRF (initial report) as soon as possible after learning of pregnancy occurrence (see [section 7.1.6, Pregnancies](#) for further details). If the subject withdraws from the study for any of the above categories except death, the site will obtain permission from the subject to continue to remain in contact with her until the outcome of the pregnancy is known, even if the outcome is not known until after the subject reaches the end of follow-up period.

Refer to [section 3.6, Stopping/Pausing Guidelines](#) and to [section 3.7 Procedures related to neutropenia](#) for further details in case of neutropenia.

3.10 End of Study

Most clinical trials intended to support the efficacy/immunogenicity and safety of an Investigational Product proceed to full completion of planned sample size accrual.

Evaluation of the primary and/or secondary immunogenicity/efficacy objectives requires the testing of biological samples from the study subjects, which can only be completed after all samples are collected. The last samples for the analysis of the primary and/or secondary objectives will be taken at visit 5. For the purpose of this protocol, end of study is defined as the completion of the testing of such biological samples, to be achieved no later than 8 months after collection of the last biological sample visit 5.

4. SELECTION OF STUDY POPULATION

4.1 Inclusion Criteria

1. Individuals ≥ 18 years to ≤ 45 years of age on the day of informed consent who are resident in the study area and are not planning to leave during the study period.
2. Individuals who, after the nature of the study has been explained, have voluntarily given written consent according to local regulatory requirements, prior to study entry.
3. Individuals who can comply with study procedures including follow-up¹.
4. Individuals in good health as determined by the outcome of medical history, physical examination, hematology, renal function, and liver function tests, urine dipstick/urinalysis and the clinical judgment of the investigator.

5. Males

Or

Females of childbearing potential who are using an effective birth control method² which they intend to use for the duration of the study

Or

Females without childbearing potential (i.e. irrespective of birth control method)

Prior to receipt of second study vaccination, subjects must be evaluated to confirm that they are eligible for subsequent vaccination. If subjects do not meet any of the original inclusion criteria listed above, they should not receive additional vaccinations.

4.2 Exclusion Criteria

1. Individuals with behavioral or cognitive impairment or psychiatric disease that, in the opinion of the investigator, may interfere with the subject's ability to participate in the study.
2. Individuals with any progressive or severe neurological disorder, seizure disorder or previous Guillain-Barré syndrome.

¹ A subject is considered to be compliant if the Investigator judges that the subject will return for all the follow-up visits as scheduled in the study.

² The following birth control methods are considered effective:

- Hormonal contraceptive (such as oral, injected or implantable contraceptives) if used for at least 2 months prior to 1st vaccination

3. Individuals who, in the judgment of the investigator, may not be able to comply with all the required study procedures.
4. Individuals with history of any illness that, in the opinion of the investigator, might interfere with the results of the study or pose additional risk to the subjects due to participation in the study.
5. Individuals with history of reactive arthritis.
6. Individuals with known HIV or hepatitis B virus infection or HIV related disease, history of an autoimmune disorder or any other known or suspected impairment /alteration of the immune system. Individuals under systemic administration of corticosteroids (PO/IV/IM) for more than 14 consecutive days within 90 days prior to screening.
7. Individuals with a known bleeding diathesis, or any condition that may be associated with a prolonged bleeding time.
8. Individuals with a neutrophil count lower than $1.8 \times 10^9/L$ (applicable to the initial 18 subjects) or lower than $1.0 \times 10^9/L$ (applicable to the additional subjects if approved by DSMB) at screening
9. Individuals with any serious chronic or progressive disease according to judgment of the investigator (e.g., neoplasm, insulin dependent diabetes, Type 2 diabetes mellitus, hypertension, cardiac, renal or hepatic disease and tuberculosis).
10. Individuals who have any malignancy or lymphoproliferative disorder.
11. Individuals with history of allergy to vaccines components or any other allergies deemed by the investigator to increase the risk of an adverse event if they were to participate in the trial.
12. Individuals participating in any clinical trial with another investigational product within 28 days prior to the screening study visit or intent to participate in another clinical study at any time during the conduct of this study.
13. Individuals who received vaccines containing meningococcal A, C, W, Y or tetanus, diphtheria or pertussis antigens within 12 months before screening, or any other vaccines within 4 weeks prior to screening in this study or who are planning to receive any vaccine within the entire study duration.
14. Individuals who have received blood, blood products, and/or plasma derivatives including parenteral immunoglobulin preparations in the 12 weeks prior to the first dose of the study vaccine.
15. Individuals who are study personnel or immediate family members (parents, children, spouse and brothers/sisters) to the personnel conducting this study.

16. Individuals with body temperature $\geq 38.0^{\circ}\text{C}$ within 3 days of intended study vaccination is a reason for delay of vaccination (see [section 4.3 Criteria for Delay of Vaccination](#) for further details).
17. Individuals with Body Mass Index (BMI) $> 30 \text{ kg/m}^2$
18. Individuals with history of substance or alcohol abuse within the past 2 years.
19. Women who are pregnant or are breast-feeding, or are of childbearing age who have not used (for the two months preceding the 1st vaccination) and are not willing to use acceptable contraceptive measures, for the duration of the study. If subjects are women of childbearing potential, they must have a negative pregnancy test at screening visit and prior to enrollment (visit 1). For the purposes of this study acceptable methods of contraception are oral, injected or implantable contraceptives.
20. Individuals who have a previously laboratory confirmed case of disease caused by *S. sonnei*.
21. Any condition which, in the opinion of the investigator, may pose an increased and unreasonable safety risk to the subject if they participated in the study.
22. Individuals with a previous history of Benign Ethnic Neutropenia or drug related neutropenia
23. Individuals who have or are likely to require concomitant treatment with neutropenic agents

Prior to receipt of second study vaccination, subjects must be evaluated to confirm that they are eligible for subsequent vaccination. If subjects meet any of the original exclusion criteria listed above, they should not receive additional vaccinations.

4.3 Criteria for Delay of Vaccination

There may be instances when individuals meet all eligibility criteria for vaccination yet have a transient clinical circumstance which may warrant delay of vaccination:

- body temperature elevation [$\geq 38.0^{\circ}\text{C}$ ($\geq 100.4^{\circ}\text{F}$) within 3 days prior to intended study vaccination]
- use of antipyretics and/or analgesic medications within 24 hours prior to vaccination.
- Individuals who have received blood, blood products and/or plasma derivatives or any parenteral immunoglobulin preparation in the past 12 weeks.

Under such circumstances, a subject may be considered eligible for study enrolment after the appropriate window for delay has passed and inclusion/exclusion criteria have been rechecked, and if the subject is confirmed to be eligible.

5. STUDY PROCEDURES

The sections that follow provide an overview of the procedures that are to be followed in enrolling, evaluating, and following subjects who participate in this clinical study. Visits can be either clinic visits or home visits, as specified in the Table below and in the [Time and Events Table 2](#).

Table 5-1 Study Procedures

Visit Category	Procedures
Pre-vaccination Clinic Visit(s)	Section 5.1 describes procedures to be followed prior to study vaccination: informed consent, screening, enrolment, and randomization
Vaccination Clinic Visit(s)	Section 5.2 describes procedures to be followed during each clinic visit involving vaccination: vaccination, post-vaccination procedures, and post-vaccination reminders
Post-vaccination Visit(s)	Section 5.3 describes follow-up clinic and home visits
Unscheduled Visit(s)	Section 5.4 describes possible procedures to be followed at unscheduled clinic visit
Study Termination Visit	Section 5.5 describes procedures to be followed at the last study visit for a subject (may include early termination visit)

5.1 Pre-vaccination Clinic Visit(s)

This section describes the procedures that must be performed for each potential subject prior to vaccination, including obtaining informed consent, screening, enrolment and randomization.

5.1.1 Informed Consent/Assent

"Informed consent" is the voluntary agreement of an individual or his/her legal guardian(s) to participate in research. Consent must be given with free will of choice, and without undue inducement. The individual must have sufficient knowledge and understanding of the nature of the proposed research, the anticipated risks and potential benefits, and the requirements of the research to be able to make an informed decision.

Informed consent following local IRB/EC guidance **must** be obtained before conducting any study-specific procedure (i.e., all of the procedures described in the protocol). The process of obtaining informed consent should be documented in the subject source document in addition to maintaining a copy of the signed and dated informed consent.

Study will be explained in either Swahili or in English, where prospective participants express a preference. If a subject is unable to read, an impartial witness should be present during the entire informed consent discussion. An impartial witness is defined as a person who is independent from study conduct, who cannot be unfairly influenced by those involved with the study, who attends the informed consent process if the subject or the subject's legally acceptable representative cannot read, and who reads the informed consent form and any other written information supplied to the subject. After the written informed consent form and any other written information to be provided to subjects, is read and explained to the subject and after the subject has verbally consented to the subject's participation in the study and, if capable of doing so, has signed and personally dated the informed consent form, the witness should sign and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the subject and that informed consent was freely given by the subject.

Pre-test counselling will be performed for individuals who consent to HIV testing. Those individuals, who will be found to be HIV positive, will be also counselled post-test and referred to the Ministry of Health HIV care services.

5.1.2 Screening

After an individual has consented to participate in the study and informed consent is signed, that individual will be given a unique 5-digit Screening Number (assigned sequentially by the site from PPD). The subject's unique Screening Number will be documented in the Screening and Enrolment log. The eligibility of the subject will be determined based on the inclusion and exclusion criteria listed in [section 4, Selection of Study Population](#) and evaluated during this screening procedure.

Prior to study enrolment, demographic data will be collected from the subject, including gender, race, body mass index.

Medical history will also be collected, including but not limited to any medical history that may be relevant to subject eligibility for study participation such as prior vaccinations, concomitant medications, and previous and ongoing illnesses or injuries. Relevant medical history can also include any medical history that contributes to the understanding of an adverse event that occurs during study participation, if it represents an exacerbation of an underlying disease/pre-existing problem.

Review of systems is a structured interview that queries the subject as to any complaints the subject has experienced across each organ system. This will be performed before enrolment and used to guide physical examination.

If applicable, prior and concomitant medications or vaccinations taken prior to start of study should be collected (refer to [section 6.5, Prior and Concomitant Medications and Vaccines](#) for further details).

Collect vital signs, heart rate, respiratory rate, blood pressure, and temperature. Measure height and weight.

Perform pregnancy testing in women of childbearing age (refer to [section 3.5, Collection of Clinical Specimens](#) for guidance regarding the procedure). Informed consent process with any women of childbearing potential will include counseling about pregnancy and family planning, including discussion of their commitment to practice acceptable birth control measures (defined as oral, injected or implantable contraceptives). Women of childbearing potential are defined as a post onset of menarche and pre-menopausal female capable of becoming pregnant. This does not include females who meet any of the following conditions: (1) menopause at least 2 years earlier, (2) tubal ligation at least 1 year earlier, (3) total hysterectomy or (4) post bilateral oophorectomy.

A general physical examination is to be performed by a qualified health care practitioner. “Qualified health care practitioner” refers to any licensed health care professional who is permitted by national policy to perform physical examinations and who is identified within the Study Staff Signature Log.

These data will be written in the source document (see [section 9.1, Source Documentation](#)).

Approximately 10 mL of blood will be drawn from all subjects for the hematology, renal and liver function testing, serological tests for HBV and HIV and pregnancy test (Refer to [section 3.5, Collection of Clinical Specimens](#) and to [synopsis Table 3, Hematological, Haematochemical Blood Tests and Urinalysis Table](#)).

In the event that the individual is determined ineligible for study participation, he/she is considered a screen failure. The reason for screen failure must be documented in the Screening and Enrolment log. If the individual is determined to be eligible for the study, he/she will be enrolled into the study.

5.1.3 Enrolment

If an individual is determined to be eligible for study participation, the investigator will enroll the subject

5.1.4 Randomization

Enrolled subjects will be randomized and automatically assigned a unique Subject ID. The Subject ID will be the subject's unique identification number for all CRFs and associated study documentation that will be used for duration of the study. After randomization, the Screening Number ceases to be used and remains in the Screening and Enrolment Log only.

At randomization, the subject will be assigned also a unique subject code. The subject code consists of the 2 letters subject's initials (the 1st letter of the surname name – followed by the 1st letter of the 1st name).

If for any reason, after signing the informed consent form (ICF), the subject who is eligible and enrolled fails to be randomized, this is called a randomization failure and the early termination study procedures must be applied. The reason for all randomization failures should be recorded in the Screening and Enrolment Log and in the source document as specified in the Source Data Agreement (SDA). The information on subjects who are randomization failures should be kept distinct from subjects who are screen failures, as described in [section 5.1.2, Screening](#).

If for any reason, after randomization the subject fails to undergo treatment, this is an Early Termination and the reason should be recorded in source document as specified in the SDA. The information on these Early Termination subjects should be kept distinct in the source documentation from randomization failures.

Screening phase will be performed until 72 subjects are randomized and vaccinated. Additional subjects may be randomized into the study at the discretion of the sponsor in the case of any subject who is randomized but does not receive any study vaccine. Subjects withdrawn or lost to follow up will not be replaced.

5.2 Vaccination Clinic Visit(s)

Vaccination will be performed on day 1 and day 29.

For studies which have visits for concomitant vaccinations or treatments, see [section 6.5, Prior and Concomitant Medications and Vaccines](#) for those visit procedures.

Ensure all serology samples are taken **prior** to each vaccination.

After completing the pre-vaccination procedures on day 1, administer the vaccine to the subject according to the procedures described in [section 6.3, Vaccine Preparation and Administration](#). Observe the blinding procedures described in [section 3.3, Blinding Procedures](#).

Prior to administration of each vaccination, confirm that the subject is eligible for additional study vaccinations and does not meet any criteria for delaying additional study vaccinations as described in [section 4, Selection of Study Population](#).

5.2.1 Post-vaccination Procedures

The following post-vaccination procedures will be performed on day 1 and day 29.

After vaccination, the subject will be observed for at least 1 hour including observation for unsolicited adverse events, solicited adverse events, and body temperature measurement. Record all safety data collected during this time in the subject's source document.

The site should schedule the next study activity (daily home visits) with the subject.

The subject should be reminded of the next planned study activity. The subject will be reminded to contact the site if there are any questions, and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical condition that leads to a hospitalization or an emergency room visit or to a visit to/by a doctor or is of concern.

5.2.2 Post-vaccination Reminders

Not Applicable

5.3 Post-vaccination Visit(s)

5.3.1 Follow-up Home Visit(s)

Post-vaccination home visits will be performed daily by a field worker from day 1 (6 hours after vaccination) to day 7 to check solicited local and systemic adverse events and medications/vaccinations received, following discussion with the subject.

A Diary Card collected in person by study personnel will be used in this study to document solicited adverse events. The Diary Card is the only source for collection of these data; therefore, it is critical that the trained site staff completes the subject's Diary Card correctly. The appropriate site staff should be trained on how and when to complete each field of subject's Diary Card.

The appropriate site staff should be trained on how to measure local solicited adverse events and body temperature. The trained field workers who will perform the home visits will be instructed to direct the subject to the clinic if the event requires medical

supervision. The measurement of solicited local adverse events is to be performed using the ruler provided by the site.

The appropriate site staff should be instructed how to perform body temperature measurement using the thermometer provided by the site. The temperature recorded in the Diary Card will be the one measured by the site staff during point of contact with subject at home.

Appropriate training should be directed at the site staff who will perform the measurements of adverse events and who will enter the information into the subject's Diary Card. The identity of the person who will enter the information into the Diary Card must be documented in the subject's source record and in the study delegation log. Any individual that makes entries into the Diary Card must receive training on completion of the Diary Card. This training must be documented.

Whenever possible, the same individual should complete the subject's Diary Card throughout the course of the study.

5.3.2 Follow-up Clinic Visit(s)

Safety follow-up clinic visits will be performed 7 days after V1 and V3.

During the follow-up clinic visit, the subject's source document and Diary Card will be reviewed. No changes to the information recorded by the trained study staff within the section of the Diary Card are permissible. For details see [sections 3.4.2, Tools Used for Data Collection](#) and [5.2.1, Post-vaccination Procedures](#). The subject will be interviewed to determine if any unsolicited adverse events occurred and if any concomitant medications or vaccines were taken/received in the time since the last clinic visit. This interview will follow a script which will facilitate the collection of relevant safety information. The healthcare professional reviewing these data will discuss the symptoms (if any) reported by the subject and will determine if any additional diagnoses and/or adverse events are present. Adverse events reported by the subject at this follow-up clinic visit must be recorded in the subject's source document and on an Adverse Events CRF, as specified in [section 7.1, Safety Assessment](#), and not written on the script used for the interview.

Perform a brief symptom-directed physical examination if necessary according to symptoms the subject has reported. This is a physical examination that will include an examination of organ systems that are relevant to the investigator based on review of the subject's reported adverse events, concomitant medication use. This assessment may include: measurement of vital signs, body temperature taken axillary and a check of general appearance. The physical assessment must be performed by the investigator or

designee of the investigator, who is qualified to perform a physical assessment in accordance with their institutional policy. Corresponding information is documented in the subject's source document and CRF(s).

At this visit blood sample for safety evaluation and urine sample for urine dipstick/urinalysis will be obtained as described in [section 3.5, Collection of Clinical Specimens](#)

The site should schedule the next study clinic visit with the subject. The subject will receive a reminder of the next planned study activity. The subject will be reminded to contact the site if there are any questions and to contact the site immediately (or as soon as the subject is medically stable) if the subject has a medical condition that leads to a hospitalization or an emergency room visit.

5.3.3 Safety Follow-up Calls

Not Applicable

5.4 Unscheduled Visits

An unscheduled visit describes a non-routine study visit triggered by a specific event. These could include anticipated or unanticipated adverse events or interventions.

All individuals with a neutropenia, occurring at any time during the study, will have additional blood draws for complete blood count to be repeated on a weekly basis until the neutropenia resolves. If neutropenia occurs at the subject's last study visit, the complete blood count will be repeated on a regular basis until resolution. For classification of neutropenia during the trial, refer to [Section 3.7, Procedures Related To Neutropenia](#).

5.5 Study Termination Visit

The study termination visit 5 will occur 28 days after 2nd vaccination. The termination visit may be a clinic visit or a home visit. The date of termination is the date of the last contact (clinic visit or home visit) in which the subject's health status was assessed or, in cases where the subject does not agree to any further safety follow-up, it is the date consent is withdrawn. This date should be recorded on the termination CRF page. For visit procedures to be performed for a subject whose planned study participation ends prematurely, please see [section 5.5.1, Early Termination Visit](#).

At the clinic visit, the following procedures will be performed:

- review of subject's medical records and source document designed to collect solicited AE,
- review of systems, interview of subject to collect unsolicited adverse events, medically attended adverse events, AEs leading to withdrawal, SAEs, AESIs, and new onset of chronic disease,
- interview of subject to collect concomitant medications/vaccinations,
- symptom-directed physical assessment including measurement of vital signs and a check of general appearance,
- Blood sampling for immunogenicity and safety laboratory assessment,
- Urine sample for urine dipstick/urinalysis and pregnancy testing.

All individuals with neutropenia either ongoing or with onset at visit 5 (last study visit) will have additional blood draws for complete blood count to be repeated on a regular basis until the neutropenia resolves (for classification of neutropenia during the trial, refer to [Section 3.7, Procedures Related To Neutropenia](#)). Laboratory results obtained after study termination will be maintained in subject medical records and not entered in the CRF.

When the clinical study report is completed, the investigators will share the summary results with the participating communities. Town hall and village meetings will be held to convey the information and to allow for questions and answers.

The site will complete the termination CRF page and this will mark the completion of the subject's participation in the study.

5.5.1 Early Termination Visit

When a subject is withdrawn from treatment or withdraws from the study, the investigator will notify the Sponsor and, when possible, will perform the procedures listed below. The reason(s) for the early termination will be included in the subject's source documentation. Early Termination Visits include subjects who were randomized but not treated.

At the clinic visit or during the home visit, the following procedures will be performed (if the Early Termination Visit is a home visit, collect as much information as possible):

- review of subject's medical records, source document and Diary Card,

- review of systems, interview of subject to collect unsolicited adverse events, medically attended adverse events, AEs leading to withdrawal, SAEs, AESIs, and new onset of chronic disease,
- interview of subject to collect concomitant medications/vaccinations,
- symptom-directed physical assessment including measurement of vital signs and a check of general appearance,
- blood sampling for safety laboratory assessment (no blood for immunogenicity assessment should be obtained unless this is agreed in advance with the sponsor),
- urine sample for urine dipstick/urinalysis and pregnancy testing.

All individuals with neutropenia either ongoing or with onset at last study visit will have additional blood draws for complete blood count to be repeated on a regular basis until the neutropenia resolves (for classification of neutropenia during the trial, refer to [Section 3.7, Procedures Related To Neutropenia](#)). Laboratory results obtained after study termination will be maintained in subject medical records and not entered in the eCRF.

The site will complete the termination CRF page and this will mark the completion of the subject's participation in the study.

6. TREATMENT OF SUBJECTS

All vaccines associated with this study are to be stored separately from other vaccines and medications in a secure location under appropriate storage conditions with temperature monitoring. **All vaccines associated with this study must be checked for expiration date prior to use. Expired vaccines must not be administered to subjects.**

6.1 Study Vaccine(s)

The term 'study vaccine' refers to those vaccines provided by the Sponsor, which will be evaluated as part of the study objectives. The study vaccines specific to this study are described below.

GVGH *S. sonnei* (1790GAHB) vaccine

The investigational agent is the GVGH *S. sonnei* vaccine. The vaccine consists of *S. sonnei* 1790-GMMA (approximately 200 µg/mL, measured by protein content) adsorbed to Alhydrogel (0.7 mg Al³⁺/mL) in Tris-buffered saline. The vaccine does not contain any preservative and is available as a liquid formulation in single dose vials with 0.7 mL of injectable solution containing approximately 140 µg of GMMA (as protein content), adsorbed onto 0.49 mg Al³⁺.

The vaccine will be used at two different antigen doses obtained by bed-side mixing. Following dilution with Alhydrogel (0.7 mg Al³⁺/mL) in Tris-buffered saline, the volume administered will be 0.5 mL for both doses:

Group A: Each 0.5 mL dose of 1790GAHB will contain approximately 25 µg of GMMA total protein and 0.35 mg of Al³⁺.

Group B: Each 0.5 mL dose of 1790GAHB will contain approximately 100 µg of GMMA total protein and 0.35 mg of Al³⁺.

In each group two vaccinations, 28 days apart, will be administered intramuscularly.

Bed-side mixing instructions will be provided to the investigator and will be located in the investigator site file.

Control vaccines

Menveo: vaccine indicated for active immunization to prevent invasive meningococcal disease caused by *Neisseria meningitidis* serogroups A, C, Y and W-135. The vaccine contains no preservative or adjuvant.

Each 0.5 mL dose of vaccine contains 10 µg MenA oligosaccharide, 5 µg of each of MenC, MenY and MenW-135 oligosaccharides conjugated to 32.7 to 64.1 µg CRM₁₉₇ protein.

Boostrix: Vaccine indicated for active booster immunization against tetanus, diphtheria, and pertussis. It contains tetanus toxoid, reduced diphtheria toxoid, and acellular pertussis antigens (inactivated pertussis toxin [PT], formaldehyde-treated filamentous hemagglutinin [FHA] and pertactin). Each antigen is individually adsorbed onto aluminum hydroxide.

For both control vaccines, the Summary of Product Characteristics (SPC) will be provided to the investigator and will be located in the investigator site file.

6.2 Non-Study Vaccines

The term ‘non-study vaccine’ refers to those vaccines which will be intentionally given to study subjects but not formally included in the analysis of study objectives.

This protocol does not foresee the use of non-study vaccines.

6.3 Vaccine Preparation and Administration

The investigator or designee will be responsible for oversight of the administration of vaccine to subjects enrolled in the study according to the procedures stipulated in this study protocol. All vaccines will be administered only by unblinded personnel who are qualified to perform that function under applicable local laws and regulations for the specific study site.

Bedside mixing and vaccine administration at the study site will be performed according to GVGH instructions and by trained site staff. The site staff responsible for these activities will be personnel who are respectively qualified according to applicable laws and regulations. GVGH will provide specific procedures and training for these activities.

PRECAUTIONS TO BE OBSERVED IN ADMINISTERING STUDY VACCINE:

Prior to vaccination, subjects must be determined to be eligible for study vaccination and it must be clinically appropriate in the judgment of the investigator to vaccinate.

Eligibility for vaccination prior to first study vaccine administration is determined by evaluating the entry criteria outlined in protocol [sections 4.1, Inclusion Criteria](#) and [4.2, Exclusion Criteria](#).

Eligibility for subsequent study vaccination is determined by following the criteria outlined in [section 4.3, Criteria for Delay of Vaccination](#).

Study vaccines should not be administered to individuals with known hypersensitivity to any component of the vaccines. Standard immunization practices are to be observed and care should be taken to administer the injection intramuscularly. Before administering vaccine, the vaccination site is to be disinfected with a skin disinfectant (e.g., 70% alcohol). Allow the skin to dry. **DO NOT inject intravascularly.**

As with all injectable vaccines, trained medical personnel and appropriate medical treatment should be readily available in case of anaphylactic reactions following vaccine administration. For example, epinephrine 1:1000, diphenhydramine, and/or other medications for treating anaphylaxis should be available.

6.4 Vaccine Administration Error or Overdose of Vaccine

Vaccine administration error is defined as receiving a dose of study vaccine that was not reconstituted as instructed or administered by a different route from the intended route of administration. An overdose of study vaccine (whether accidental or intentional) is defined when a dose higher than the recommended dose is administered in one dose of study vaccine.

An overdose would also occur if two doses of the study vaccine are administered within half the time of the recommended interval between doses, as defined in the protocol i.e. for this protocol, both an overdose and vaccine administration error would occur if receipt of more than one dose takes place in a 12 day period.

Any vaccine administration error or overdose of study vaccine detailed in this protocol must be reported as an adverse event, and if the vaccine administration error or overdose is associated with a serious adverse event, it must be reported as such within 24 hours to the Sponsor.

6.5 Prior and Concomitant Medications and Vaccines

All medications, vaccines and blood products taken or received by the subject within 4 weeks prior to the start of the study are to be recorded on the Prior and Concomitant Medications CRF.

The use of antipyretics and/or analgesic medications within 24 hours prior to vaccination must be identified and the reason for their use (prophylaxis versus treatment) must be described in the source document and Concomitant Medications CRF. Use of antipyretics/analgesics within 24 hours prior to vaccine administration is a reason to delay study vaccination (see [section 4.3, Criteria for Delay of Vaccination](#)).

Medications taken for prophylaxis are those intended to prevent the onset of symptoms. Medications taken for treatment are intended to reduce or eliminate the presence of symptoms that are present.

Concomitant medications include all medications (including vaccines) taken by/administered to the subject at and after enrolment and must be documented on the Concomitant Medications CRF.

When recording concomitant medications/vaccines, they should be checked against the study entry and continuation criteria in [section 4, Selection of Study Population](#) to ensure that the subject should be enrolled/continue in the study.

6.6 Vaccine Supply, Labeling, Storage and Tracking

The Sponsor will ensure the following:

- Supply the study vaccine(s).
- Appropriate labeling of all study vaccines provided that complies with the legal requirements of each country where the study is to be performed. Possibly necessary additional labelling of clinical study materials at the study site will be performed according to GVGH instructions and by trained site staff who are qualified according to applicable laws and regulations.

The investigator must ensure the following:

- Acknowledge receipt of the study vaccines by a designated staff member at the site, including:
 - Confirmation that the vaccines were received in good condition
 - Confirmation to the Sponsor of the temperature range during shipment from the Sponsor to the investigator's designated storage location
 - Confirmation by the Sponsor that the vaccines are authorized for use.
- Proper storage of the study vaccines, including:
 - Storage in a secure, locked, temperature-controlled location.
 - Proper storage according to the instructions specified on the labels.
 - Appropriate record keeping and inventory of the study vaccines, including regular documentation of adequate storage temperature.
- Appropriate use of the study vaccines, including:
 - Not use of vaccines prior to receipt of authorization for use from the Sponsor.

- Use only in accordance with the approved protocol.
- Dilution in accordance to bed-side mixing procedure and documentation
- Proper handling, including confirmation that the vaccine has not expired prior to administration.
- Appropriate documentation of administration of vaccines to study subjects including:
 - Date, dosage, batch/lot numbers, expiration dates, unique identifying numbers assigned to subjects and study vaccines, and time of vaccine administration. This information will be maintained in an accountability log that will be reviewed by the site monitor.
 - Reconciliation of all vaccines received from the Sponsor. Reconciliation is defined as maintaining records of which and how many vaccines were received, which vaccines (and dilution for *S. sonnei* vaccine) were administered to subjects, which vaccines were destroyed at the site, and which vaccines were returned to the Sponsor, as applicable.
- Proper adherence to the local institutional policy with respect to destruction of study vaccines.
- Complete record keeping of vaccine use, wastage, return or destruction, including documentation of:
 - Copy of the site's procedure for destruction of hazardous material.
 - Number of doses destroyed, date of destruction, destruction code (if available), method of destruction, and name of individual performing destruction.

Vaccines that have been stored differently from the manufacturer's indications **must not** be used unless the Sponsor provides written authorization for use. In the event that the use cannot be authorized, the Sponsor will make every effort to replace the vaccine supply. All vaccines used in conjunction with this protocol must be stored separately from normal hospital/practice stocks to prevent unintentional use of study vaccines outside of the clinical study setting.

Monitoring of vaccine accountability will be performed by the study monitor during site visits and at the completion of the study.

At the conclusion of the study, and as appropriate during the course of the study, the investigator must ensure that all unused study vaccines, packaging and supplementary labels are destroyed locally (upon approval from Sponsor) or returned to the Sponsor.

7. ASSESSMENTS

7.1 Safety Assessment

The measures of safety used in this study are based on previous study data. They include a close vigilance for, and stringent reporting of selected local and systemic adverse events routinely monitored in vaccine studies as indicators of reactogenicity.

An adverse event (AE) is defined as any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product at any dose that does not necessarily have to have a causal relationship with this treatment. Therefore, an AE can be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the investigational product. This definition includes intercurrent illnesses or injuries and exacerbation of pre-existing conditions.

The period of observation for AEs extends from the time the subject signs informed consent until he or she completes the specified safety follow-up period (28 days after each vaccination) or terminates the study early (whichever comes first). AEs occurring after the informed consent form is signed but prior to receiving study vaccine/product will be documented as an adverse event and recorded within source document. However, any AEs occurring prior to receipt of any study vaccine will be analyzed separately from “treatment emergent” AEs (AEs occurring after administration of the first study vaccine).

Adverse events are collected as either solicited or unsolicited adverse events. Solicited events are derived from organized data collection systems, such as Subject interview.

7.1.1 Solicited Adverse Events

The term “reactogenicity” refers to solicited signs and symptoms (“solicited adverse events”) occurring in the hours and days following a vaccination, to be collected by the subject for 7 consecutive days.

The following solicited adverse events are included in the Diary Card that will be used in this study to document solicited adverse events.

Solicited local adverse events: erythema, induration and pain at injection site.

Solicited systemic adverse events: headache, arthralgia, chills, fatigue, malaise, myalgia, and fever (body temperature measured axillary).

Other solicited reactions: Use of analgesics/antipyretics, body temperature (measured axillary).

Each adverse event is to be assessed using the scoring system reported below:

Solicited Local Adverse Events

Solicited local adverse events	Grade 0 Absent	Present - Grading of Severity		
		Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe
Injection site Erythema (Captured as measurements in millimeters)	1-24 mm	25-50 mm	51-100 mm	> 100 mm
Injection site Induration (Captured as measurements in millimeters)	1-24 mm	25-50 mm	51-100 mm	> 100 mm
Injection site Pain	No pain	Present but does not interfere with activity	Interferes with activity	Prevents daily activity

Solicited Systemic Adverse Events

Solicited systemic adverse events	Grade 0 Absent	Present - Grading of Severity*		
		Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe
Headache	No headache	Present but does not interfere with activity	Interferes with activity	Prevents daily activity
Arthralgia	No arthralgia	Present but does not interfere with activity	Interferes with activity	Prevents daily activity
Chills	No chills	Present but does not interfere with activity	Interferes with activity	Prevents daily activity
Fatigue	No fatigue	No interference with activity	Some interference	Significant; prevents daily

			with activity	activity
Malaise	No malaise	No interference with activity	Some interference with activity	Significant; prevents daily activity
Myalgia	No myalgia	Present but does not interfere with activity	Interferes with activity	Prevents daily activity
Fever as a Body temperature (Captured as measurements in degrees Celsius)	$\leq 37.9^{\circ}\text{C}$	$\geq 38.0 - 38.9^{\circ}\text{C}$	$\geq 39.0 - 39.9^{\circ}\text{C}$	$\geq 40.0^{\circ}\text{C}$
	Axillary Temperature: $<35.5^{\circ}\text{C}$ to $\geq 38.0^{\circ}\text{C}$			

Other Solicited Adverse Events

Other Solicited adverse events	Grade 0 Absent	Present - Grading of Severity		
		Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe
Use of analgesics/antipyretics	Categorized as “yes” or “no”			

The study staff must review the data entered into the Diary Card as described in [section 3.4.2, Tools Used for Data Collection](#) and [section 5.3.1, Follow-up Clinic Visit\(s\)](#).

Note: Any solicited adverse event that meets any of the following criteria must be entered into subjects’ source document (see [section 9.1, Source Documentation](#)) and also as an adverse event on the Adverse Event CRF:

- Solicited local or systemic adverse event that continues beyond day 7 after vaccination.
- Solicited local or systemic adverse event that leads to a visit to a healthcare provider (medically attended adverse event, see [section 7.1.3, Evaluation of Adverse Events](#)).
- Solicited local or systemic adverse event leading to the subject withdrawing from the study or the subject being withdrawn from the study by the investigator (adverse event leading to withdrawal, see [section 7.1.3, Evaluation of Adverse Events](#)).
- Solicited local or systemic adverse event that otherwise meets the definition of a serious adverse event (see [section 7.1.4, Serious Adverse Events](#)).

7.1.2 Unsolicited Adverse Events

An unsolicited adverse event is an adverse event that was not solicited using the Diary Card and that was spontaneously communicated by a subject who has signed the informed consent.

Potential unsolicited AEs may be medically attended (defined as symptoms or illnesses requiring hospitalization, or emergency room visit, or visit to/by a health care provider), or were of concern to the subject. In case of such events, subjects will be instructed to contact the site as soon as possible to report the event(s). The detailed information about the reported unsolicited AEs will be collected by the qualified site personnel during the interview and will be documented in the subject's records.

Unsolicited AEs that are not medically attended nor perceived as a concern by subjects will be collected during interview with the subject and by review of available medical records at the next visit (see section 5.3, [Post-vaccination Visit\(s\)](#)).

7.1.3 Evaluation of Adverse Events

Every effort should be made by the investigator to evaluate safety information reported by a subject for an underlying diagnosis and to capture this diagnosis as the event in the AE page. In other words, the practice of reporting only symptoms (e.g., "cough" or "ear pain") are better reported according to the underlying cause (e.g., "asthma exacerbation" or "otitis media").

The severity of events reported on the Adverse Events CRF will be determined by the investigator as:

Mild:	transient with no limitation in normal daily activity.
Moderate:	some limitation in normal daily activity.
Severe:	unable to perform normal daily activity.

The relationship of the study treatment to an AE will be determined by the investigator based on the following definitions:

1. Not Related

The AE is not related to an investigational vaccine if there is evidence that clearly indicates an alternative explanation. If the subject has not received the vaccine, the timing of the exposure to the vaccine and the onset of the AE are not reasonably related in time, or other facts, evidence or arguments exist that reasonably suggest an alternative explanation, then the AE is not related.

2. Possibly Related

The administration of the investigational vaccine and AE are considered reasonably related in time and the AE could be explained by exposure to the investigational vaccine or by other causes.

3. Probably Related

Exposure to the investigational vaccine and AE are reasonably related in time and no alternative explanation has been identified.

The relationship of the study treatment to an unsolicited AE will be determined by the investigator.

Note: solicited AEs will not be evaluated for relationship to study treatment. Grading for severity of solicited local and systemic AEs is described in [section 7.1.1, Solicited Adverse Events](#).

Adverse events will also be evaluated by the investigator for the co-existence of any of the other following conditions:

- “Medically attended adverse event”: an adverse event that leads to a visit to a healthcare provider.
- “New onset of chronic disease” (NOCD): an adverse event that represents a new diagnosis of a chronic medical condition that was not present or suspected in a subject prior to study enrolment.
- AEs leading to withdrawal: adverse events leading to study or vaccine withdrawal.

If solicited or unsolicited adverse events have been reported and the subject indicated that the symptoms required medical attendance or were of concern, the subject must be contacted for further information.

When the subject is contacted for any of these reasons, the contact must be documented in the subject’s source documentation.

All AEs, regardless of severity, will be monitored until resolution or until the investigator assesses them as chronic or stable. All subjects experiencing AEs - whether considered associated with the use of the study vaccine or not - must be monitored until symptoms subside and any abnormal laboratory values have returned to baseline, or until there is a satisfactory explanation for the changes observed, or until death, in which case a full pathologist’s report should be supplied, if possible. The investigator’s assessment of

ongoing Adverse Events at the time of each subject's last visit should be documented in the subject's medical chart.

7.1.4 Serious Adverse Events

A serious adverse event (SAE) is defined as any untoward medical occurrence that at any dose results in one or more of the following:

- Death.
- Is life-threatening (i.e., the subject was, in the opinion of the investigator, at immediate risk of death from the event as it occurred); it does not refer to an event which hypothetically might have caused death if it were more severe.
- Required or prolonged hospitalization.
- Persistent or significant disability/incapacity (i.e., the event causes a substantial disruption of a person's ability to conduct normal life functions).
- Congenital anomaly/or birth defect.
- An important and significant medical event that may not be immediately life threatening or resulting in death or hospitalization but, based upon appropriate medical judgment, may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above.

Adverse events which do not fall into these categories are defined as non-serious.

It should be noted that a severe adverse event need not be serious in nature and that a serious adverse event need not, by definition, be severe.

Serious adverse events will be captured both on the Vaccines Serious Adverse Event (VSAE) form as well as on the AE CRF. All SAEs will be evaluated by the investigator for relationship of the event to study vaccine. SAEs that are judged to be possibly or probably related to the study vaccine should be reported to the Sponsor as related/suspected events.

The relationship of the study treatment to an SAE will be determined by the investigator based on the following definitions:

1. Related/suspected

The SAE is judged by the investigator to be possibly or probably related to the study vaccine on the AE CRF page (see [section 7.1.3, Evaluation of Adverse Events](#)).

2. Not Related

The SAE is not related if exposure to the study vaccine has not occurred, **or** the occurrence of the SAE is not reasonably related in time, **or** the SAE is considered unlikely to be related to use of the study vaccine, i.e., there are no facts (evidence) or arguments to suggest a causal relationship.

The relationship of the study vaccine to an SAE will be determined by the investigator.

In addition, SAEs will be evaluated by the Sponsor or designee for “expectedness.” An unexpected AE is one that is not listed in the current Summary of Product Characteristics or the Investigator’s Brochure or an event that is by nature more specific or more severe than a listed event.

In addition, a pre-existing event or condition that results in hospitalization should be recorded on the Medical History CRF. If the onset of an event occurred before the subject entered the study (e.g., any pre-planned hospitalization for conditions like cosmetic treatments or for non-emergency routine visits for a pre-existing condition), the hospitalization would not lead to an AE being classified as serious unless, in the view of the investigator, hospitalization was prolonged as a result of participation in the clinical study or was necessary due to a worsening of the pre-existing condition.

7.1.4.1 Adverse Events of Special Interest

Adverse events of special interest (AESIs) are predefined adverse events that will be specifically highlighted to the investigator and will be summarized separately at the end of the study. All AESIs occurring during the study will be categorized and reported as a SAE.

Reactive arthritis (ReA) will be collected and analyzed as an AESI for this study.

Reactive arthritis is defined as non-purulent joint inflammation that develops in response to an infection in another part of the body. Since the inflammation is triggered by a previous condition, it is termed “reactive”. Intestinal pathogens that have been associated with reactive arthritis include *Campylobacter*, *Salmonella*, *Yersinia*, *Clostridium difficile*, and *Shigella*. If reactive arthritis is caused by an auto immune response, there is at least a possibility that it could be initiated by vaccination of susceptible people with the 1790GAHB vaccine.

For diagnosis of ReA, imaging and aspiration are not required (unless clinically indicated).

Neutropenia, a decrease of circulating neutrophils below the lower limit of the range of normality, will be collected and analyzed as an AESI for this study. To this purpose, as specified in the study procedures, subjects in this trial will have multiple blood draws for monitoring potential episodes of post vaccination neutropenia. For additional information on neutropenia, please refer to Section 3.7, Procedures Related To Neutropenia

7.1.5 Methods for Recording Adverse Events and Serious Adverse Events

Findings regarding Adverse Events must be reported on an Adverse Events CRF, as specified in [section 7.1.1, Solicited Adverse Events](#), and on the VSAE form, if applicable, which is part of the Investigator Site File. All findings in subjects experiencing AEs must be reported also in the subject's source document.

All SAEs which occur during the course of the study, whether considered to be associated with the study vaccination or not, must be reported **within 24 hours of the site becoming aware of the event** to GVGH or its designee. Specific instructions and contact details for collecting and reporting SAEs to GVGH will be provided to the investigator.

All SAEs are also to be documented on the Adverse Events CRF. Any medication or other therapeutic measures used to treat the AE will be recorded on the appropriate CRF(s) in addition to the outcome of the AE.

After receipt of the initial report, representatives of GVGH or its designee will contact the investigator if it is necessary to obtain further information for assessment of the event.

All SAEs must be reported by the investigator to his/her IRB or applicable regulatory authorities in accordance with institutional policy/regulatory requirements and adequate documentation of this notification must be provided to the Sponsor.

GVGH or its designee must also comply with the applicable regulatory requirement(s) related to the reporting of suspected unexpected serious adverse vaccine reactions (also known as SUSARs) to the regulatory authority(ies) and the IRB/EC. If a SUSAR or other safety signal relating to use of one of the study vaccines is reported to GVGH or its designee, the Sponsor will communicate the information to the investigator and the investigator will be responsible for submitting this information to the IRB and other relevant authorities.

7.1.5.1 Post-Study Events

Any SAE that occurs outside of the protocol-specified follow-up period and considered to be caused by the study vaccine must be reported to GVGH or its designee. These SAEs will be processed by GVGH or its designee as during the course of the study, until 6

months after last subject last visit. Instructions and contact details for collecting and reporting these suspected SAEs will be provided to the investigator.

7.1.6 Pregnancies

To ensure subjects' safety, each pregnancy in a subject after study vaccination must be reported to GVGH or delegate within 72 hours of the site learning of its occurrence. If the subject agrees to submit this information, the pregnancy must be followed to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. This follow-up should occur even if intended duration of safety follow-up for the study has ended.

Pregnancy data must be recorded on a Pregnancy Report CRF (initial report) and Pregnancy Follow-Up CRF (outcome report) and reported to GVGH or delegate. Instructions and contact details for submitting the Pregnancy CRFs will be provided to the investigator.

Any pregnancy outcome meeting the definition of a SAE (see [section 7.1.4, Serious Adverse Events](#)) must also be reported on the VSAE Report Form.

7.1.7 Safety Laboratory Measurements

For list of safety laboratory measurement, refer to synopsis [Table 3, Hematological, Haematochemical Blood Tests and Urinalysis Table](#).

Safety laboratory measurement will be performed as described in [Section 3.5, Collection of Clinical Specimens](#).

Significant alterations in hematology, blood chemistry and urinalysis will be clinically assessed by the investigator's medical judgment based on interpretation of deviations from institution's normal values.

Any abnormality in laboratory measurements classified as clinically significant must be reported in the Adverse Event CRF form.

If a subject is to have blood drawn and/or urine testing for safety laboratory for repeat safety assessment (e.g., in case of markedly abnormal safety laboratory parameter), investigator's medical judgment will be applied concerning values that would trigger reanalysis and frequency of repeats.

Safety laboratory assessments will be performed at the site laboratory, and results of these tests will be recorded in the source documents and in the CRF.

7.2 Efficacy Assessment

This study has no efficacy measurements.

7.3 Immunogenicity Assessment

The measure of immunogenicity used in this study i.e. IgG enzyme-linked immunosorbent assay (ELISA) in sera is standard, widely used and generally recognized as reliable, accurate, and relevant (able to describe the quality and extent of the immune response). The ELISA methodology used in this study has been adopted based on scientific consensus and has been deemed appropriate to describe the immune response against *Shigella sonnei* GMMA in this study.

Testing will be conducted by a GVGH or designated laboratory in a blinded manner towards the treatment arm and the visit.

The measure of immunogenicity used in this study is IgG ELISA against *Shigella sonnei* OAg. The serologic assays will be conducted on serum samples and will be performed by a GVGH or designated laboratory in a blinded manner towards the treatment arm and the visit.

For reference of visits the measurements are taken, refer to [section 3.5](#) and to the Clinical Specimen Laboratory Manual.

8. STATISTICAL CONSIDERATIONS

8.1 Endpoints

8.1.1 Primary Endpoint(s)

8.1.1.1 Primary Safety Endpoint(s)

The safety profile of the two vaccine doses and control(s) will be assessed through measurement of the following:

- a. Number and percentage of subjects with solicited local and systemic adverse reactions during 7 days following each vaccination.
- b. Numbers and percentage of subjects with deviations from normal ranges of safety laboratory data after each vaccination.
- c. Number and percentage of subjects with reported unsolicited adverse events during 28 days following each vaccination.
- d. Number and percentage of subjects with reported SAEs throughout the study duration.
- e. Number and percentage of subjects with reported reactive arthritis or neutropenia (AESIs).

8.1.1.2 Primary Efficacy Endpoint(s)

The study does not have primary efficacy endpoint(s).

8.1.1.3 Primary Immunogenicity Endpoint(s)

The study does not have primary immunogenicity endpoint(s).

8.1.2 Secondary Endpoint(s)

8.1.2.1 Secondary Safety Endpoint(s)

The study does not have secondary safety endpoint(s).

8.1.2.2 Secondary Efficacy Endpoint(s)

The study does not have secondary efficacy endpoint(s),

8.1.2.3 Secondary Immunogenicity Endpoint(s)

The measures of the immunogenicity outcome, (i.e., the anti-LPS *S. sonnei* serum IgG), will include:

- a. IgG Geometric mean concentrations (GMCs) pre-vaccination (Day 1), 28 days after 1st vaccination and 28 days after 2nd vaccination, as determined by Enzyme-linked Immunosorbent Assay (ELISA), and applicable geometric mean ratios between post- and pre-vaccination samples.
- b. Number and percentage of subjects with seroresponse for anti- LPS *S. sonnei* at 28 days after 1st vaccination and 28 days after 2nd vaccination

Seroresponse is aimed to define a significant increase in post vaccination samples based on the biological performance of this specific serology assay and it is defined as:

- If the baseline value is greater than 50 ELISA Units (EU) then an increase of at least 50% in the post-vaccination sample as compared to baseline [i.e. ((Post-vac minus baseline)/baseline)100% \geq 50%]
 - If the baseline value is less or equal to 50 EU then an increase of at least 25 EU in the post-vaccination sample as compared to baseline [i.e. (post-vac minus baseline) \geq 25 EU]
- c. Number and percentage of subjects with titers post vaccination concentration \geq 121 for anti-LPS *S. sonnei* at 28 days after 1st vaccination and 28 days after 2nd vaccination
 - d. IgG Geometric mean concentrations (GMCs) pre-vaccination (Day 1), 28 days after 1st vaccination and 28 days after 2nd vaccination by antibody titer at baseline (i.e., above vs. below the assay detection limit), as determined by Enzyme-linked Immunosorbent Assay (ELISA), and applicable geometric mean ratios between post- and pre-vaccination samples.
 - e. Number and percentage of subjects with seroresponse for anti- LPS *S. sonnei* at 28 days after 1st vaccination and 28 days after 2nd vaccination by antibody titer at baseline (i.e., above vs. below the assay detection limit).

A post vaccination concentration \geq 121 anti-LPS serum IgG units in the GVGH ELISA corresponds to a titer of 1:800 in the ELISA method used by Cohen et al. (Cohen et al., 1989). This antibody level is the median antibody concentration of a set of 87 convalescent subjects previously infected by *S. sonnei*. The value of 121 anti-LPS serum IgG units in the Novartis GVGH ELISA was determined by calibration against the Cohen

ELISA (i.e., the GVGH standard serum was tested in Cohen's lab using the Cohen's methodology)

8.1.3 Exploratory Endpoint(s)

8.1.3.1 Exploratory Safety Endpoint(s)

The study does not have exploratory safety endpoint(s).

8.1.3.2 Exploratory Efficacy Endpoint(s)

The study does not have exploratory efficacy endpoint(s).

8.1.3.3 Exploratory Immunogenicity Endpoint(s)

Other assays (including serum secretory IgA) might be done to further characterize the immune response to the study vaccine.

8.2 Success Criteria

This is a Phase 2a trial and there is no pre-defined success criterion.

8.2.1 Success Criteria for Primary Objective(s)

Not applicable.

8.2.1.1 Success Criteria for Primary Safety Objective(s)

Not applicable.

8.2.1.2 Success Criteria for Primary Efficacy Objective(s)

Not applicable.

8.2.1.3 Success Criteria for Primary Immunogenicity Objective(s)

Not applicable. The study does not have primary immunogenicity endpoint(s).

8.2.2 Success Criteria for Secondary Objective(s)

Not applicable.

8.2.2.1 Success Criteria for Secondary Safety Objective(s)

Not applicable. The study doesn't have secondary safety objectives.

8.2.2.2 Success Criteria for Secondary Efficacy Objective(s)

Not applicable. The study doesn't have secondary efficacy objectives.

8.2.2.3 Success Criteria for Secondary Immunogenicity Objective(s)

This is a Phase 2a trial and there are no pre-defined success criteria for secondary immunogenicity objectives.

8.3 Analysis Sets

8.3.1 All Enrolled Set

All screened subjects who provide informed consent and provide demographic and/or baseline screening assessments, regardless of the subject's randomization and treatment status in the study and received a Subject ID (where the first two digits identify the site number)

8.3.2 All Exposed Set

All subjects in the enrolled set who receive a study vaccination.

8.3.3 Safety Set

Solicited Safety Set (solicited local and systemic adverse events and other solicited adverse events)

All subjects in the Exposed Set with any solicited adverse event data and/or indicators of solicited adverse events (e.g., use of analgesics/antipyretics).

Unsolicited Safety Set (unsolicited adverse events)

All subjects in the Exposed Set with unsolicited adverse event data.

Overall Safety Set

All subjects who are in the Solicited Safety Set and/or Unsolicited Safety Set.

Subjects will be analyzed as "treated" (i.e., according to the vaccine a subject received, rather than the vaccine to which the subject may have been randomized).

8.3.4 Full Analysis Set (FAS) Immunogenicity Set

Full Analysis Set Immunogenicity

All subjects in the All Enrolled Set who are randomized, receive at least one study vaccination and provide immunogenicity data at data at relevant time points.

- Received at least one study vaccination and provide immunogenicity data 28 days after 1st vaccination. (FAS 1 - day 28 after 1st vaccination)
- Received at least one study vaccination and provide immunogenicity data 28 days after 2nd vaccination. (FAS 2 - day 28 after 2nd vaccination)

In case of vaccination error, subjects in the FAS sets will be analyzed “as randomized” (i.e., according to the vaccine a subject was designated to receive, which may be different from the vaccine the subject actually received).

8.3.5 Per Protocol (PP) Set Immunogenicity Set

All subjects in the FAS Immunogenicity who:

- Correctly receive the vaccine (i.e., receive the vaccine to which the subjects is randomized and at the scheduled time points).
- Have no protocol deviations leading to exclusion (see [section 8.3.8, Protocol Deviations](#)) as defined prior to unblinding.
- Are not excluded due to other reasons defined prior to unblinding or analysis (see [section 8.3.8, Protocol Deviations](#))

PPS are subsets of FAS and should be always defined even if the objectives do not require it.

Examples for subjects excluded due to other reasons than protocol deviations are:

- Subjects who withdrew informed consent.

8.3.6 Other Analysis Sets

Modified FAS

The differences between FAS and Modified FAS are:

- Subjects who received the same wrong vaccine at the two vaccinations will be analyzed in the vaccine the subject actually received (in the FAS they will be analyzed according to the vaccine the subject was designed to receive)
- If a subject didn't receive the vaccination but a blood sample after the vaccination visit (where subject didn't receive the vaccination) was collected then subject will be excluded from the immunogenicity analysis for all visits after the vaccination visit.

8.3.7 Subgroups

Not applicable.

8.3.8 Protocol Deviations

A protocol deviation is any change, divergence, or departure from the study design or procedures of a study protocol. A protocol deviation may be a reason to remove data from an analysis set at the time of analysis. CSR-reportable protocol deviations will be defined as exclusionary from the analysis according to protocol objectives and endpoints, which will be specified in the statistical analysis plan. In some cases exclusion of data may be due to a reason other than a protocol deviation, e.g. early termination.

8.4 Statistical Analysis Plan

8.4.1 Analysis of Demographic and Baseline Characteristics

Descriptive statistics (mean, standard deviation, median, minimum and maximum) for, age, height and weight and BMI at enrolment will be calculated overall and by vaccine group.

Distributions of subjects by sex and ethnic origin will be summarized overall and by vaccine group.

8.4.2 Analysis of Primary Objective(s)

8.4.2.1 Analysis of Primary Safety Objective(s)

The analysis of safety assessments in this study will include summaries of the following categories of safety data collected for each subject:

- Vaccine exposure.
- Solicited local and systemic adverse events.
- Unsolicited adverse events.

- Clinical Laboratory Investigations.

8.4.2.1.1 Analysis of Extent of Exposure

The frequencies and percentages of subjects with vaccinations will be summarized overall and by vaccine group. Data will be tabulated for the All Enrolled Set.

8.4.2.1.2 Analysis of Solicited Local, Systemic and Other Adverse Events

All solicited adverse events will be summarized according to defined severity grading scales.

Frequencies and percentages of subjects experiencing each adverse event will be presented for each symptom severity. Summary tables showing the occurrence of any local or systemic adverse event overall and at each time point will also be presented.

Post-vaccination solicited adverse events reported from day 1 to day 7 will be summarized for the intervals day 1-3, day 4-7, day 1-7 by maximal severity and by vaccine group. The severity of solicited local adverse events, including injection-site erythema and induration will be summarized according to categories based on linear measurement: 25 to 50 mm, 51 to 100 mm, >100mm.

Injection site pain and systemic adverse events (except fever) occurring up to 7 days after each vaccination will be summarized according to “mild”, “moderate” or “severe”.

Each solicited local and systemic adverse event will also be further summarized as “none” versus “any”.

Implausible measurements (for further definition see statistical analysis plan) will be left out of the analysis.

Use of antipyretics and analgesics will be summarized by frequency by type of use (prophylactic versus treatment) and percentage of subjects reporting use.

Body temperature will be summarized by 0.5 °C increments from 36.0 °C up to ≥ 40 °C and will be broken down according by route of measurement.

8.4.2.1.3 Analysis of Unsolicited Adverse Events

This analysis applies to all adverse events occurring during the study, judged either as probably related, possibly related, or not related to vaccination by the investigator, recorded in AE CRF, with a start date on or after the date of first vaccination. AE starting prior to the first vaccination will only be listed. The original verbatim terms used by

investigators to identify adverse events in the CRFs will be mapped to preferred terms using the MedDRA dictionary. The adverse events will then be grouped by MedDRA preferred terms into frequency tables according to system organ class (SOC).

All reported adverse events, as well as adverse events judged by the investigator as at least possibly related to study vaccine, will be summarized according to SOC and preferred term within SOC. These summaries will be presented by vaccination group. When an adverse event occurs more than once for a subject, the maximal severity and strongest relationship to the vaccine group will be counted.

Separate summaries will be produced for the following categories:

- Serious adverse events and adverse events of special interest.
- Adverse events that are possibly or probably related to vaccine.
- Adverse event leading to withdrawal.

Data listings of all adverse events will be provided by subject. In addition, adverse events in the categories above will be provided as listed data.

8.4.2.1.4 Analysis of Safety Laboratory Values

The investigator must assess all safety laboratory results ([see section 7.1.7](#)). Clinically significant modifications in blood chemistry, hematology, and urinalysis test values will be assessed by medical judgment based on interpretation of deviations from the institution's normal values.

All laboratory safety data will be analyzed descriptively by vaccine group. Safety laboratory data will be shown in a 3 x 3 table by visit using categorization of laboratory values of hematological and haematochemical blood tests and urinalysis according to institutional normal reference range (below, within, above).

8.4.2.2 Analysis of Primary Efficacy Objective(s)

This study does not include primary efficacy objectives.

8.4.2.2.1 Statistical Hypotheses

Not applicable.

8.4.2.2.2 Analysis Sets

Not applicable.

8.4.2.2.3 Statistical Methods

Not applicable.

8.4.2.3 Analysis of Primary Immunogenicity Objective(s)

This study does not have primary immunogenicity objectives.

8.4.2.3.1 Statistical Hypotheses

Not applicable.

8.4.2.3.2 Analysis Sets

Not applicable.

8.4.2.3.3 Statistical Methods

Not applicable.

8.4.3 Analysis of Secondary Objective(s)

8.4.3.1 Analysis of Secondary Safety Objective(s)

This study does not have secondary safety objectives.

8.4.3.1.1 Analysis of Extent of Exposure

Not applicable.

8.4.3.1.2 Analysis of Solicited Local, Systemic and Other Adverse Events

Not applicable.

8.4.3.1.3 Analysis of Unsolicited Adverse Events

Not applicable.

8.4.3.1.4 Statistical Hypotheses

Not applicable.

8.4.3.1.5 Analysis Sets

Not applicable.

8.4.3.1.6 Statistical Methods

Not applicable.

8.4.3.2 Analysis of Secondary Efficacy Objective(s)

This study does not have secondary efficacy objectives.

8.4.3.2.1 Statistical Hypotheses

Not applicable.

8.4.3.2.2 Analysis Sets

Not applicable.

8.4.3.2.3 Statistical Methods

Not applicable.

8.4.3.3 Analysis of Secondary Immunogenicity Objective(s)

8.4.3.3.1 Statistical Hypotheses

This Phase 2a safety and immunogenicity study is aimed to descriptively evaluate the safety and immunogenicity profiles of the study vaccines. No specific hypotheses are tested in this study.

8.4.3.3.2 Analysis Sets

The modified FAS will be the primary analysis set for the immunogenicity objective.

8.4.3.3.3 Statistical Methods

Analysis of continuous variables

The ELISA concentrations will be logarithmically transformed (base10) (to fulfil the normal distribution assumption). GMC will be calculated, with their associated two-sided 95% CIs, by exponentiating the corresponding log-transformed means and their 95% CI.

Additionally, within-subject GMRs will be computed for GMTs/GMCs at one month after first and second vaccination versus baseline (day 1). The GMRs and 95% CIs will be constructed by exponentiating the mean within-subject differences in log-transformed titers and the corresponding 95% CIs.

Analysis of binary variables

The number and percentages of subjects with seroresponse from baseline and with high antibody level after vaccination (i.e. post vaccination antibody level ≥ 121 IgG units in the GVGH ELISA), will be summarized. Two-sided 95% Clopper-Pearson CIs for the percentages will be computed.

Titers below the limit of detection will be set to half that limit for the purposes of analysis. Missing values of immunogenicity will be excluded from analyses (i.e. complete-case analysis) since they are considered missing completely at random, i.e. not informative and with no impact on inferences.

8.4.4 Analysis of Exploratory Objectives

Other assays, including serum secretory IgA, might be done to further characterize the immune response to the study vaccine. The analysis will be described in the statistical analysis plan.

8.4.4.1 Analysis of Exploratory Safety Objective(s)

Not applicable.

8.4.4.2 Analysis of Exploratory Efficacy Objective(s)

Not applicable.

8.4.4.3 Analysis of Exploratory Immunogenicity Objective(s)

Not applicable.

8.5 Sample Size and Power Considerations of Primary Objectives

No formal statistical sample size and power computations are performed since the objectives of the study are to descriptively assess the immunogenicity and safety of the investigational vaccine.

8.6 Interim Analysis

For up to 45 subjects, once they have completed the study, a group unblinded preliminary immunogenicity analysis and a blinded interim safety analysis will be performed. Individual subject results from preliminary analyses will not be made available to site and sponsor personnel until the end of the study. The results of these immunogenicity and safety analyses will not impact the conduct of the study, but will inform the planning of future studies with the same vaccine.

9. SOURCE DOCUMENTATION, STUDY MONITORING AND AUDITING

Study monitoring and auditing will be standardized and performed in accordance with the Sponsor's or delegated contract research organization's (CRO) standard operating procedures and applicable regulatory requirements (e.g., FDA, EMA, and ICH guidelines).

Prior to enrolment of the first study subject, GVGH or delegate will train investigators and/or their study staff on the study protocol, all applicable study procedures, documentation practices and all electronic systems. CRFs supplied by the Sponsor must be completed for each enrolled subject (see [section 8.3.1, All Enrolled Set](#) for definition of enrolled subject). Documentation of screened but not enrolled subjects must be maintained at the site and made available for review by the site monitor. Data and documents will be checked by the Sponsor and/or monitor.

9.1 Source Documentation

Prior to the start of the study, the site staff participating in the study conduct will be instructed on what documents will be required for review as source documents. The kinds of documents that will serve as source documents will be agreed between Sponsor or delegate and investigator and designees and specified in the SDA prior to subject enrolment.

In addition, source documentation **must** include all of the following: subject identification (on each page), eligibility and participation, proper informed consent procedures, dates of visits, adherence to protocol procedures, adequate reporting and follow-up of adverse events, documentation of prior/concomitant medication/vaccines, study vaccine receipt/dispensing/return records, study vaccine administration information, any data collected by a conversation with the subject and date of completion and reason.

The subject must also allow access to the subject's medical records. Each must be informed of this prior to the start of the study and consent for access to medical records may be required in accordance with local regulations.

All safety data reported by subjects must be written down in source documents prior to entry of the data into CRFs. If there are multiple sources of information (e.g., verbal report of the subject, telephone contact details, medical chart) supporting the diagnosis of an adverse event, these sources must be identified in the source documents, discrepancies between sources clarified, the ultimate diagnosis must be justified and written in the source documents, and this diagnosis must be captured in the Adverse Event CRF (AE CRF). The AE CRF must also capture which source(s) of information were used to determine the adverse event (e.g., subject recall, medical chart).

9.2 Study Monitoring, Auditing and Source Data Verification

Prior to enrolment of the first study subject, GVGH or its designee (e.g., a CRO) will develop a Clinical Monitoring Plan (or comparable documentation) to specify how centralized and/or on-site monitoring, including clinical specimens reconciliation, will be performed for the study. Study progress will be monitored by GVGH or its designee as frequently as necessary to ensure:

- that the rights and well-being of human subjects are protected,
- the reported study data are accurate, complete, and verifiable from the source documents and
- the conduct of the study is in compliance with the current approved protocol/amendment(s), GCP and applicable regulatory requirements.

Contact details for the GVGH team or its designee involved in study monitoring will be provided to the investigator. Study data recorded on CRFs will be verified by checking the CRF entries against source documents in order to ensure data completeness and accuracy as required by study protocol except for those parameters which are specifically described in [section 7, Assessment](#) being entered directly into the EDC system.

Data verification may also be performed through a centralized review of data (e.g., checking for outliers or other anomalies). Additional documents such as the investigator site file, pharmacy records, and informed consent documentation must also be available for review if requested. Arrangements for monitoring visits will be made in advance in accordance with the monitoring plan, except in case of emergency.

The investigator and/or site staff must make source documents of subjects enrolled in this study available for inspection by GVGH or its representative at the time of each monitoring visit and Sponsor audits, when applicable. These documents must also be available for inspection, verification and copying, as required by regulations, by officials of the regulatory health authorities (e.g., FDA, EMA and others) and/or ECs/IRBs. The investigator and study site staff must comply with applicable privacy, data protection and medical confidentiality laws for use and disclosure of information related to the study and enrolled subjects.

10. DATA MANAGEMENT

10.1 Data Entry and Management

In this study, all clinical data (including, but not limited to, AE/SAEs, concomitant medications, medical history, and physical assessments), safety data, and immunogenicity data will be entered onto case report forms (CRFs) in a timely fashion by the investigator and/or the investigator's dedicated site staff. Data entered onto CRFs are stored on a secure website. The data collected on this secure website are assimilated into an electronic data capture (EDC) system, which is compliant with Title 21 Part 11 policies of the Code of Federal Regulations ([FDA 1997](#)). The data system includes password protection and internal quality checks. The EDC system will be designed and validated by the Sponsor prior to activation for data entry by sites. The investigator or designated delegate must review data entered and electronically sign the CRFs to verify their accuracy.

Access to the EDC system for data entry or review will require training and distinct individual access code assignments to those site staff members who will be entering study data and those involved in study oversight who may review study data. Data are collected within the EDC system, to which the Sponsor and site monitors have exclusively "read only" access.

10.2 Data Clarification

As part of the conduct of the trial, the Sponsor may have questions about the data entered by the site, referred to as queries. The monitors and the Sponsor are the only parties that can generate a query. All corrections and clarifications will be entered into the EDC system and will be identified by the person entering the information, the reason for the change, as well as the time of the changes made. If changes are made to a previously and electronically signed CRF, the investigator must confirm and endorse the changes.

10.3 Data Protection

GVGH respects the subjects' rights to privacy and will ensure the confidentiality of their medical information in accordance with all applicable laws and regulations.

11. RECORD RETENTION

Investigators must retain all study records required by GVGH and by the applicable regulations in a secure and safe facility. The investigator must consult a GVGH representative before disposal of any study records, and must notify the Sponsor of any change in the location, disposition, or custody of the study files. Essential documents must be retained for 15 years. “Essential documents” are defined as documents that individually and collectively permit evaluation of the conduct of a study and the quality of the data produced. These documents should be retained for a longer period, however, if required by the applicable national regulatory or institutional requirements.

These principles of record retention will also be applied to the storage of laboratory samples, provided that the integrity of the stored sample permits testing.

12. USE OF INFORMATION AND PUBLICATION

GVGH assures that the key design elements of this protocol will be posted in a publicly accessible database such as clinicaltrials.gov, and in compliance with current regulations.

GVGH also assures that key results of this clinical study will be posted in a publicly accessible database within the required time-frame from the end of study as defined in [section 3.9, End of Study](#).

In accordance with standard editorial, ethical practices and current guidelines of Good Publication Practice ([Graf 2009](#)), GVGH will generally support publication of multicenter studies only in their entirety and not as individual center data. In this case, a coordinating investigator will be designated by mutual agreement prior to the start of the study. The coordinating investigator will also sign the clinical study report on behalf of the principal investigators ([CPMP/EWP/2747/00](#)). Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements. Any formal publication of the study in which contribution of GVGH personnel exceeded that of conventional monitoring will be considered as a joint publication by the investigator and the appropriate GVGH personnel.

GVGH must be notified of any intent to publish data collected from the study and prior approval from GVGH must be obtained prior to submission for publication.

13. ETHICAL CONSIDERATIONS

13.1 Regulatory and Ethical Compliance

The study will be conducted in compliance with the protocol, GCP and applicable regulatory requirement(s).

This clinical study was designed and shall be implemented and reported in accordance with the ICH Harmonized Tripartite Guidelines for Good Clinical Practice, with applicable local regulations and with the ethical principles laid down in the Declaration of Helsinki ([European Council 2001](#), [US Code of Federal Regulations](#), [ICH 1997](#)).

For each scheduled study visit (Screening visit and Visit 1 to 5) subjects will be reimbursed Kenyan Shillings (KSh) 1,000 per visit as follows:

- KSh 500 for daily lost wages and time spent at study clinic;
- KSh 300 for the cost of transportation;
- KSh 200 for food.

In case of acute illness, subjects will receive free medical care. Subjects who will develop chronic conditions will be referred to the respective Ministry of Health clinic for further management and follow-up.

13.2 Informed Consent Procedures

Eligible subjects may only be included in the study after providing written informed consent, as described in [section 5.1.1, Informed Consent/Assent](#). Before the start of the study, the investigator will have the informed consent and any other materials that will be provided to the subjects reviewed and approved by the IRB/EC. This review and approval will be documented and stored with other study documents. The investigator or designee must fully inform the subject or legal guardian of all pertinent aspects of the study. A copy of the written informed consent will be given to the subject or the designee. The subject/designee must be allowed ample time to ask about the details of the study and to make a decision as to whether or not to participate in the study. The subject **must** sign the consent form indicating their agreement to participate in the study before any study-related procedures are conducted. If the subject is unable to read and write, a witness must be present during the informed consent discussion and at the time of informed consent signature.

Prior to the start of the study, GVGH will provide to investigators a proposed informed consent form that complies with the ICH GCP guideline and regulatory requirements and is considered appropriate for this study. Any changes to the proposed consent form suggested by the investigator must be agreed to by GVGH before submission to the IRB/EC and a copy of the approved version must be provided to the GVGH monitor after IRB/EC approval.

Women of childbearing potential should be informed that taking the study medication may involve unknown risks to the fetus if pregnancy were to occur during the study and agree that in order to participate in the study they must adhere to the contraception requirements indicated in the protocol for the duration of the study. If case of doubts on the ability of a subject to adhere to these requirements, that subject should not be allowed in the study.

13.3 Responsibilities of the Investigator and IRB/EC

The protocol and the proposed informed consent form must be reviewed and approved by a properly constituted IRB/EC before study start. Properly constituted IRB/EC is defined in ICH Guideline for Good Clinical Practice E6 (R1), Section 3 ([ICH 1997](#)). A signed and dated statement that the protocol and informed consent have been approved by the IRB/EC must be given to GVGH before study initiation. Prior to study start and at any time the protocol is amended during study conduct, the investigator is required to sign a protocol signature page confirming his/her agreement to conduct the study in accordance with these documents and all of the instructions and procedures found in this protocol and to give access to all relevant data and records to GVGH monitors, auditors, GVGH Clinical Quality Assurance representatives, designated agents of GVGH, IRBs/ECs, and regulatory authorities as required. If an inspection of the clinical site is requested by a regulatory authority, the investigator must inform GVGH immediately that this request has been made.

The investigator also responsible for the following:

- Maintaining a list of appropriately qualified persons to whom the investigator has delegated significant study-related duties.
- Demonstrating the capability of recruiting the required number of suitable subjects within the recruitment period.
- Demonstrating sufficient time and staffing to properly conduct and complete the study within the agreed study period.
- Ensuring that all persons assisting with the study are adequately informed about the protocol, the investigational product(s), and their study-related duties and functions

- Ensuring that appropriately trained health care professionals are responsible for all study-related medical decisions and for ensuring appropriate medical care of subjects experiencing any adverse event related to the study.

The investigator should not implement any deviation from, or changes of the protocol without agreement by the Sponsor and prior review and documented approval/favourable opinion from the IRB/IEC of an amendment, except where necessary to eliminate an immediate hazard(s) to study subjects, or when the change(s) involves only logistical or administrative aspects of the study (e.g., change in monitor(s), change of telephone number(s)). In addition, the investigator, or person designated by the investigator, should document and explain any deviation from the approved protocol.

The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to study subjects without prior IRB/IEC approval/favourable opinion. As soon as possible, the implemented deviation or change, the reasons for it, and, if appropriate, the proposed protocol amendment(s) should be submitted:

- (a) to the IRB/IEC for review and approval/favourable opinion,
- (b) to the Sponsor for agreement and, if required,
- (c) to the regulatory authority(ies).

13.4 Protocol Amendments

An amendment is a written description of change(s) to or formal clarification of a study protocol which may impact on the conduct of the clinical study, potential benefit of the clinical study, or may affect subject safety, including changes of study objectives, study design, subject population, sample sizes, study procedures, or significant administrative aspects. An administrative change of a study protocol is a minor correction or clarification that has no significant impact on the way the clinical study is to be conducted and no effect on subject safety (e.g., change of telephone number(s), logistical changes). Protocol amendments must be approved by GVGH, health authorities where required, and the IRB/EC. In cases when the amendment is required in order to protect the subject safety, the amendment can be implemented prior to IRB/EC approval. Notwithstanding, the need for formal approval of a protocol amendment, the investigator is expected to take any immediate action required for the safety of any subject included in this study, even if this action represents a deviation from the protocol. In such cases, GVGH should be notified of this action, the IRB/EC at the study site, and, if required by local regulations, the relevant health authority) should be informed within 10 working days.

14. REFERENCE LIST

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