STATISTICAL ANALYSIS PLAN

Study 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
Study Number:	H03_04TP
Protocol Version and Date:	Version 3.0 of 27 May 2016
Phase of Development:	Phase 1
Plan Prepared by:	PPD
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Approvers:	PPD MD, Head GVGH CD&RA

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The attached Analysis Plan has been prepared by:

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LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Events of Special Interest
ANOVA	Analysis of Variance
BCDM	Biostatistics and Clinical Data Management
CRA	Clinical Research Associate
CSR	Clinical Study Report
CTL	Clinical Trial Leader
DSMB	Data Safety Monitoring Board
FAS	Full Analysis Set
GMC	Geometric Mean Concentration
GMMA	Generalized Modules for Membrane Antigens
GMR	Geometric Mean Ratio
HCO	SBVGH Head of Clinical Operations
MedDRA	Medical Dictionary for Regulatory Activities
GVGH	GSK Vaccines Institute for Global Health
PD	Protocol Deviation
PPS	Per Protocol Set
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SP	Statistical Programmer
SUSAR	Suspected Unexpected Serious Adverse Reactions
TFL	Tables, Figures and Listings
TOC	Table of Content
WBR	Web-based Randomization

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 Organizations (WHO) estimated approximately 125 million cases of shigellosis per year in Asia alone. Ninety-nine percent of all cases occur in developing countries and approximately 70% in children younger than 5 years of age. 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 with Shigella sonnei being the most common serotype worldwide.

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-trimoxasole. Resistance to third generation antibiotics, especially ciprofloxacin, has been reported to be emerging [3]. Still effective antibiotics include ceftriaxone that is administered intramuscularly or intravenously and thus is not easily accessible for people in impoverished communities.

No vaccine is available. The GVGH candidate vaccine against shigellosis caused by *Shigella sonnei* (1790GAHB) has been developed with a novel technology based on high yield production of Generalized Modules for Membrane Antigens (GMMA) from *S. sonnei*. GMMA are outer membrane particles naturally released from the *S. sonnei* during growth.

This GVGH *S. sonnei* 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.

For further details please refer to section 1.0 of the protocol.

2. OBJECTIVES

Primary Objective:

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

Secondary Objective:

To evaluate the immunogenicity profile of two injections of *S. sonnei* 1790GAHB vaccine in healthy adults by measuring the anti-LPS S. *sonnei* serum IgG.

3. 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 1st injection and one dose of Boostrix as 2nd 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 3-1 Overview of the Study Groups

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

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 1st injection and 7 and 28 days after 2nd injection) and daily home visits performed for 6 days after the 1st and 2nd injection.

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.

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 ≥ 38.0°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.

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).

For further details please refer to section 3.0 of the protocol.

Table 3-1: Times 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 in the study protocol**								
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

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 in the study protocol**								
Post Injection Assessment[e]	Sections 5.1.2		X			X			
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)**

a. Informed Consent to be obtained before any study procedure
b. 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.
c. Compliance with Exclusion/Inclusion criteria should be verified.
d. 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.

e. 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.

4. RANDOMIZATION AND BLINDING

4.1 Method of Group Assignment and Randomization

At Visit 1, enrolled subjects will be randomly assigned to one of the 3 vaccine groups.

The list of randomization assignments is produced by a validated system used by the GSK Biostatistics and Clinical Data Management (BCDM) department. The validated web-based system automates the random assignment to dosage and treatment groups according to the subject numbers.

For further details please refer to section 5.1.4 'Randomization' of the protocol.

4.1.1 Definition of Randomization/Vaccination Errors

The list below provides some examples of potential errors that may occur during vaccination:

- Subjects got vaccinated with a vaccine different from the one assigned at randomization (PD code 120)
- Subjects got vaccinated with the correct vaccine but containing a lower volume (PD code 140.3)
- Subjects got vaccinated by route of administration different from the one assigned at randomization

Differentiate between incorrect stratification and misrandomizations.

A misrandomization is defined as a subject receiving a vaccine other than the one assigned by randomization. Misrandomization is a Clinical Study Report (CSR)-reportable Protocol Deviation (PD) and should be analyzed as randomized in Full Analysis Set (FAS), excluded from Per Protocol Set (PPS) and analyzed as received for Safety.

Incorrect stratification is defined as enrollment and randomization of a subject based on incorrect stratification information at baseline. Incorrect stratifications should be split for major and minor errors.

- Major stratification errors: resulting in administration of incorrect dosage or schedule, not corrected on time, i.e. not corrected before administration of the vaccine. These will be handled as CSR-reportable PD.
- Minor stratification errors: not having impact on dose/schedule administered. These will *not* be considered as CSR reportable PD.

Stratification error	FAS	PPS	Safety Set
Minor	Analyze as corrected	Analyze as corrected	Analyze as corrected
Major	Analyze as originally stratified	Exclude from analysis	Analyze as corrected

Please see section 7 of this document for a complete guidance on how vaccination errors are handled in the statistical analysis.

4.1.2 Forced Randomization

Not applicable.

4.2 Blinding and Unblinding

For details please refer to section 3.3 'Blinding Procedure' of the protocol.

If a subject is unblinded during the study, it is to be reported as CSR-reportable PD (PD code 130), except for subjects unblinded by Pharmacovigilance due to suspected unexpected serious adverse reactions (SUSAR). The unblinding will be documented appropriately.

The unblinded subject(s) are excluded from the PPS. The unblinded subjects will be included in the FAS and safety sets.

Further details on measures taken to ensure blinding can be found in the study-specific Data Security Plan.

5. SAMPLE SIZE AND POWER CONSIDERATIONS

For details please refer to section 8.5 'Sample Size and Power Considerations of Primary Objectives' of the protocol.

Sample size/power considerations are included in the study protocol.

6. DETERMINATION OF PROTOCOL DEVIATIONS

6.1 Definition of Protocol Deviations

CSR reportable PD are defined in accordance with ICH E3 as important PDs related to study inclusion or exclusion criteria, conduct of the trial, subject management or subject assessment resulting in the potential to jeopardize the safety or rights of the trial subjects or the scientific value of the trial.

All reportable PDs will be evaluated before unblinding and classified according to ICH into the following five categories:

- Subject developed withdrawal criteria during the study but was not withdrawn
  - Underlying medical condition forbidden by the protocol or which may influence immune response (PD code 240).
  - Subject had contraindication for a subsequent study vaccination but was vaccinated (PD code 220).
  - Concomitant infection related to the vaccine which may influence immune response (PD code 250).
- Subject received wrong vaccine or incorrect dose
  - Study vaccine was not administered at all (PD code 100)
  - Vaccine administration not according to protocol (PD code 140).
  - Randomization failure (vaccination not according to randomization) (PD code 120).
- Subject took an excluded concomitant medication
  - Administration of concomitant vaccine(s) forbidden in the protocol (PD code 150).
  - Administration of any medication forbidden by the protocol (PD code 230).
- Subject randomized and did not satisfy the entry criteria
  - Subject did not meet entry criteria (PD code 200).
- Deviation from key study procedures
  - Randomization code was broken (PD code 130).
  - Subject did not comply with study vaccination schedule (PD code 140.x).

- Subject did not provide any post-vaccination unsolicited safety data (PD code 115).
- Subject did not provide any post-vaccination solicited safety data (PD code 116).
- Subject did not comply with blood draw schedule (PD code 270).
- Serological results not available post-vaccination (PD code 110).
- Obvious incoherence, abnormal serology evolution or error in data (PD code 112).

CSR reportable PDs will lead to exclusion of the subject or part of the subject's data from at least one analysis set.

The number of subjects in any and by PD category will be summarized by vaccine, and overall. Individual subject listings will be provided in an appendix, sorted by subject and by PD category.

Prior to unblinding, designated GVGH staff will develop a memo that describes the PDs that led to exclusions from analysis sets. This memo will be signed off by at least the Biostatistician and the Physician and will be included in the trial master file (Exclusion Memo).

Prematurely terminating study participation for reasons such as withdrawal of consent or occurrence of adverse events (including death) is not considered as a PD. The missing assessments that should have otherwise been collected for that subject later in the study are also not considered as a PD.

6.2 Determination of Protocol Deviations

All protocol deviations will be tracked by using the protocol deviations log according to GVGH SOP MON2403. Protocol deviations will be provided to the GVGH Project Physician (PP) and GVGH Head of Clinical Operations (HCO) for review, on an ongoing basis during the study.

GVGH Project Physician, Clinical Research Associate (CRA) and HCO will provide the Biostatistician with:

- An assessment of CSR reportable PDs based on blinded clinical data review.
- An assessment of subjects without PDs (e.g., premature withdrawals due to adverse event, withdrawal of consent) who should be excluded from an analysis set.

6.3 Exclusions of Individual Values for Safety Analysis

Some local and systemic adverse events will be directly measured by the subject and will not be subject to a reconciliation process, even if they are biologically implausible. Therefore these implausible measurements will be removed from the analysis but included in listings. Implausible measurements are summarized in the table below:

Table 6.3-1: Implausible Solicited Adverse Events

Parameter	Implausible measurements
Body temperature	≤ 33°C or ≥ 42°C
Erythema	For subjects ≥ 6 years: ≥ 900 mm Measurements < 0 mm
Induration	For subjects ≥ 6 years: ≥ 500 mm Measurements < 0 mm

7. ANALYSIS SETS

7.1 All Enrolled Set

All screened subjects who provide informed consent and provide demographic and/or other baseline screening measurements, regardless of the subject's randomization and vaccination status in the trial, and receive a subject ID (starting with 01).

Demography and baseline characteristics tables as well as subject listings will be produced on the All Enrolled Set.

7.2 Exposed Set

All subjects in the All Enrolled Set who receive a study vaccination.

7.3 Full Analysis Set (FAS), Immunogenicity Set

All subjects in the All Enrolled Set who are randomized, receive at least one study vaccination and provide immunogenicity 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 vac)

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

In case of vaccination error, subjects in the FAS 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).

If a subject is unblinded during the study, he/she will be included in the FAS.

7.4 Per Protocol Set (PPS), 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 CSR-reportable PD leading to exclusion (see section 6.2) as defined prior to unblinding.
- Are not excluded due to other reasons defined prior to unblinding (see section 6.2)

In case of vaccination error, the subject is excluded from the PPS. If a subject receives a vaccine, labelled for another subject but the same as the one the subject was randomized to, the subject will not be removed from the PPS.

If a subject received the correct study vaccine (dose, batch) but from another ongoing study at the site, then the subject should be excluded from the PPS.

If a subject is unblinded during the study (except for SUSAR), he/she will be excluded from the PPS.

Summary tables will display the number of subjects available in the different analyses sets.

7.5 Safety Set

Solicited Safety Set

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)].

For the by vaccination tabulations, solicited safety sets will be defined as follows:

- Solicited safety set #1 for post-injection reactions after the first dose (SOL 1)
- Solicited safety set #2 for post-injection reactions after the second dose (SOL 2)

Unsolicited Safety Set

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

In this respect, a confirmation of no AE is considered as adverse event data; hence subject is to be included.

Overall Safety Set

All subjects who are in the solicited safety set and/or in the unsolicited safety set.

In case of vaccination error, subjects will be analyzed as "treated" (i.e., according to the vaccine a subject receives, rather than the vaccine to which the subject is randomized).

If a subject received the correct study vaccine (dose, batch) but from another ongoing study at the site then the subject's safety data should be included in the safety analysis.

If a subject is unblinded during the study, he/she will be included in all the safety sets.

7.5.1 Restricted Safety Set

Not applicable.

7.6 Other Analysis Set

Modified FAS

The difference between FAS and Modified FAS are:

Subjects who received a wrong vaccine at both 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)

7.7 Overview of Analysis Sets by PD Code

Table 7.7-1: Safety Sets

PD code	PD Description	Study Objective/ Period	All Exposed Set	Overall Safety Set	Safety Set, Unsolicited AEs, All study period	Safety Set, Solicited AEs, Period 1, T30-D7	Safety Set, Solicited AEs, Period 2, T30-D7
	Exclusion code		**EXPFL**	**SAFFL**	**SSU10FL**	**SSS10FL**	**SSS20FL**
100	**Study vaccine not administered AT ALL**	**All Study**	EXC	EXC	EXC	EXC	EXC
115	**Subject did not provide any post-vaccination unsolicited safety data – All study period**	**All Study**			EXC		
116	**Subject did not provide any post-vaccination solicited safety data**	**All Study**				EXC	EXC
116.1	**Subject did not provide any post-vaccination solicited safety data after 1st vaccination**	**Period 1**				EXC	
116.2	**Subject did not provide any post-vaccination solicited safety data after 2nd vaccination**	**Period 2**					EXC

EXC = excluded from this analysis set.

Table 7.7-2: Immunogenicity Sets

PD code	PD Description	Study Objective/ Period	All Exposed	FAS 1 - day 28 after 1st vac	FAS 2 - day 28 after 2nd vac	PPS X -	PPS 1 - day 28 after 1st vac	PPS 2 - day 28 after 2nd vac
	Exclusion code		**EXPFL**	**FAS10FL**	**FAS20FL**	**PPSX0FL**	**PPS10FL**	**PPS20FL**
100	**Study vaccine not administered AT ALL**	**All Study**	EXC	EXC	EXC	EXC	EXC	EXC
110	**Serological results are not available**	**All Study**		EXC	EXC	EXC	EXC	EXC
110.3	**Serological results are not available for visit 3 (post 1st vac)**	**Visit 3**		EXC		EXC	EXC	
110.5	**Serological results are not available for visit 5 (post 2nd vac)**	**Visit 5**			EXC			EXC
112	**Obvious deviation from Laboratory Manual or error in laboratory data**							
112.3	**Incoherence between CRF and CLS database in terms of sample availability at visit 3**	**Visit 3**				EXC	EXC	

PD code	PD Description	Study Objective/ Period	All Exposed	FAS 1 - day 28 after 1st vac	FAS 2 - day 28 after 2nd vac	PPS X -	PPS 1 - day 28 after 1st vac	PPS 2 - day 28 after 2nd vac
	Exclusion code		**EXPFL**	**FAS10FL**	**FAS20FL**	**PPSX0FL**	**PPS10FL**	**PPS20FL**
112.5	**Incoherence between CRF and CLS database in terms of sample availability at visit 5**	**Visit 5**						EXC
120	**Randomization failure**		EXC	EXC	EXC	EXC	EXC	EXC
120.2	**Subject received another vaccine than allocated (Actual Arm different from Planned Arm) at 1st vacc**	**All study**		EXC	EXC	EXC	EXC	EXC
130	**Randomization code was broken**	**All study**				EXC	EXC	EXC
140	**Vaccination not according to protocol**					EXC	EXC	EXC
140.1	**Administration of temperature-deviated vaccine**					EXC	EXC	EXC

PD code	PD Description	Study Objective/ Period	All Exposed	FAS 1 - day 28 after 1st vac	FAS 2 - day 28 after 2nd vac	PPS X -	PPS 1 - day 28 after 1st vac	PPS 2 - day 28 after 2nd vac
	Exclusion code		**EXPFL**	**FAS10FL**	**FAS20FL**	**PPSX0FL**	**PPS10FL**	**PPS20FL**
140.2	**Administration of expired vaccine**					EXC	EXC	EXC
140.5	**Incomplete vaccination series**					EXC	EXC	EXC
150	**Administration of forbidden vaccine between Visit X and Visit Y**	**Visit X – Visit Y**				EXC		
200	**Subject did not meet entry criteria**	**All study**				EXC	EXC	EXC
260	**Did not comply with study vaccination schedule**					EXC		
260.2	**2nd vaccination not done or performed out of planned visit window**							EXC
270	**Did not comply with blood draw schedule**							

PD code	PD Description	Study Objective/ Period	All Exposed	FAS 1 - day 28 after 1st vac	FAS 2 - day 28 after 2nd vac	PPS X -	PPS 1 - day 28 after 1st vac	PPS 2 - day 28 after 2nd vac
	Exclusion code		**EXPFL**	**FAS10FL**	**FAS20FL**	**PPSX0FL**	**PPS10FL**	**PPS20FL**
270.3	**Blood draw at visit 3 not done or performed out of planned visit window**	**Visit 3**					EXC	
270.5	**Blood draw at visit 5 not done or performed out of planned visit window**	**Visit 5**						EXC

FAS = Full Analysis Set; PPS=Per Protocol Set; M=Month EXC = excluded from this analysis set.

8. GENERAL ISSUES FOR STATISTICAL ANALYSES

8.1 Adjustment for Covariates

Not applicable.

Summary tables will show unadjusted GMCs for each vaccine group and unadjusted ratios of GMCs.

Binary data tables will show unadjusted percentages.

8.2 Handling of Dropouts, Missing Data

First-line analyses will be without missing values.

8.2.1 Safety Data

Solicited adverse events: The solicited study period will be '30 min - day7'.

No imputation methods will be used to address missing values.

Unsolicited adverse events: The unsolicited adverse events will be analyzed in the entire study period.

8.2.2 Immunogenicity Data

Missing immunogenicity values are considered MCAR and therefore will not contain information that impact the result of the analysis (i.e., not informative). Imputation methods will therefore not be used. The immunogenicity objectives will be analyzed using the modified FAS.

8.2.3 Efficacy Data

Not applicable.

8.3 Multicenter Studies

Not applicable as this is a single center study.

To be included in section 11.4.2.4 of the CSR and not part of the SAP. Subjects were recruited in one center only.

8.4 Multiple Comparisons and Multiplicity

Statistical analyses will only be used for descriptive purposes therefore no multiplicity adjustment will be done.

To be included in section 11.4.2.5 of CSR and not part of the SAP.

Statistical analyses were only used for descriptive purposes therefore no multiplicity adjustment was made.

8.5 Immunogenicity Subsets

Not applicable. All subjects will be evaluated for the immunogenicity analysis.

8.6 Subgroups

Not applicable.

8.7 Data Transformation

Distributions of antibodies are generally skewed to the right (Nauta, 2010). Therefore, prior to any statistical analysis that assumes normally distributed observations, antibody titers or concentrations will be $\log_{10}$-transformed. GMTs [/GMCs] and their 95% CIs are computed by exponentiating (base 10) the least squares means and 95% CIs of the $\log_{10}$ titers.

8.8 Derived and Computed Variables

Demographics

Age will be calculated in years using the following formula:

(Date of Visit 1 – Date of Birth + 1) / 365.25

Body Mass Index (kg/m^2) will be calculated using the following formula:

Weight (kg) / Height^2 (m^2)

Immunogenicity

Values below the limit of quantification (recorded as "< LQ") will be set to half that limit (i.e., LQ/2]).

Seroresponse is defined for subjects with non-missing values pre-vaccination and post-vaccination as:

If the baseline value is greater than 50 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% ≥ 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) ≥ 25 EU]

High seroresponse is defined for subjects with non-missing post-vaccination as:

Post vaccination titer ≥ 121 anti-LPS serum IgG units in the GSK ELISA

Geometric Mean Concentration

The GMC will be calculated using the following formula:

$$10^{\left\{\frac{\sum_{i=1}^{n}\log_{10}(t_i)}{n}\right\}}$$

where $t_1, t_2, \ldots, t_n$ are n observed immunogenicity titers/concentrations.

Geometric Mean Ratio

Geometric mean ratios (GMRs) measure the changes in immunogenicity titers/concentrations *within* subjects.

The GMR will be calculated using the following formula:

$$10^{\left\{\frac{\sum_{i=1}^{n}\log_{10}\left(v_{ij}/v_{ik}\right)}{n}\right\}} = 10^{\left\{\frac{\sum_{i=1}^{n}\log_{10}(v_{ij})-\log_{10}(v_{ik})}{n}\right\}}$$

where, for n subjects, v_{ij} and v_{ik} are observed immunogenicity titers/concentrations for subject i at time-points j and k, $j \neq k$.

Duration in the Study

Duration in the study is defined in days as:

Last visit date (visit x)[a] – Enrollment date (visit 1) + 1

[a]or premature discontinuation date (in case of withdrawal from the study)

The duration is missing if one of the dates is missing or incomplete.

Solicited Adverse Events

For details see section 13.2.

Unsolicited Adverse Events

All adverse events will be characterized according to the date of occurrence related to the vaccination phase as follows:

- **Emergence before vaccination phase**: start date before the first date of injection of study vaccine.
- **Emergence during vaccination phase**: start date on or after the first date of injection of study vaccine or, adverse event increase in severity including to "serious" adverse event.

If start date is equal to the first date of injection then "timing" variable ("On injection day, before injection"/"On injection day, after injection") will be used to define whether the adverse event occur before or after the injection.

If there are several vaccinations, the adverse event will be associated with the most recent vaccination.

If an adverse event start date is missing or unknown, the adverse event will be considered as emergent.

When start and/or end dates of an adverse event are only partially known, adverse events will be categorized as emergent before, during, or after vaccination phase using the following rules:

- If the partial end date is before (<) the first study vaccination (i.e., year or year & month is/are before the first study vaccination year or year & month) then the adverse event is emergent before vaccination phase.
- If the partial start date is equal or after (≥) the first study vaccination (i.e., year or year & month is/are after or the same as the first study injection year or year & month) then the adverse event is emergent during vaccination phase.

The **maximum event severity** is the greatest severity associated with a preferred term for a reported adverse event according to the following order: Mild < Moderate < Severe. Unknown/ Missing severity is considered as severe (except for the definition of emergence).

Multiple AEs with the same PT for the same subject are counted only once.

Vaccination-related Adverse Events are those for which the cause has been evaluated by the investigator, and recorded either as possibly related, probably related or unknown/ missing.

Safety Laboratory Data

All laboratory measurements summarized in the CSR are those defined in the study protocol.

Reference ranges used to categorize the results as "low" (values below the lower limit of the reference range), "normal" (values within the reference range) or "high" (values above the upper value of the reference range) will be those provided by the site lab which performed the tests and provided the laboratory reports.

Prestudy, Concomitant and Post-Vaccination Medications

A **previous medication** is a medication used only before the first study vaccination (i.e. medication end date < first study vaccination date).

All other medications are **concomitant**.

When start and/or end dates of a medication intake are missing, the medication is considered as concomitant with the study vaccination schedule.

If the first study vaccination date is missing then the medication is considered as concomitant with the study vaccination schedule, provided that the study vaccine was administered to the subject.

For further details please refer to the technical and program specifications document stored in Home/analysis/H03_01TP/final/prod/docs within the SAS Drug Development (SDD) server.

8.9 Analysis Software

All analyses will be performed using SAS Software version 9.2 or higher.

9. STUDY SUBJECTS

9.1 Disposition of Subjects and Withdrawals

All subjects who provide informed consent will be accounted for in this study. The frequencies and percentages of subjects in each analysis set, study withdrawals, subgroups, and major protocol deviations will also be presented.

10. DEMOGRAPHICS AND OTHER BASELINE CHARACTERISTICS

10.1 Demographics

Age, height, weight, body mass index will be summarized by reporting the mean, standard deviation, median and range, and will be calculated by vaccine group and overall.

The frequencies and percentages of subjects by sex, country, age categories (for posting), ethnic origin, race, entry criteria fulfilled will be presented by vaccine group and overall. Demographic data will be tabulated for the All Enrolled, modified FAS and Safety sets.

10.2 Medical History

The frequencies and percentages of subjects with medical history will be presented by MedDRA system organ class and preferred term, by vaccine group and overall. Medical history data will be tabulated for the All Enrolled.

11. IMMUNOGENICITY ANALYSIS

11.1 Blood Samples

The frequencies and percentages of subjects with blood draws will be summarized overall and by vaccine group. Data will be tabulated for the enrolled set.

11.2 Primary Objectives Analysis

Not Applicable. The primary objective of the study is safety and all immunogenicity objectives are secondary objectives.

11.3 Secondary Objectives Analysis

Secondary Objective:

To evaluate the immunogenicity profile of two injections of *S. sonnei* 1790GAHB vaccine in healthy adults by measuring the anti-LPS S. *sonnei* serum IgG.

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% ≥ 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) ≥ 25 EU]

c. Number and percentage of subjects with titers post vaccination concentration ≥ 121 for anti-LPS *S. sonnei* at 28 days after 1st vaccination and 28 days after 2nd vaccination

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.

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 ≥ 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 *S. sonnei*. 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).

Statistical hypothesis:

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

Statistical models:

GMCs

The ELISA concentrations will be logarithmically transformed (base10) (to fulfil the normal distribution assumption). For each treatment group, 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 and with high seroresponse in ELISA concentrations from baseline, 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.

11.4 Exploratory Objectives Analysis

Explorative Objective:

There are no exploratory objectives in this trial.

12. EFFICACY ANALYSIS

12.1 Primary Objectives Analysis

Not Applicable.

12.2 Secondary Objectives Analysis

Not Applicable.

12.3 Exploratory Objectives Analysis

Not Applicable.

13. SAFETY ANALYSIS

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 and indicators of solicited adverse events.
- Unsolicited adverse events.
- Clinical Laboratory Investigations.

13.1 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.

13.1.1 Safety Completeness Analysis

Solicited adverse events

The safety completeness analysis on solicited adverse events aims to identify subjects who completed diary cards, irrespective of severity will not be done for this Phase 2a study.

13.2 Solicited Local and Systemic Adverse Events

For details please refer to section 6.5 of the protocol.

All solicited local and systemic adverse events will be analyzed. Implausible measurements will not be taken into consideration in the analysis but listed in the Appendix (see section 6.3).

Solicited adverse events will be reported at 30 minutes, 2 hours, 6 hours on day 1 and then daily until day 7 using structured diaries. The analyses of solicited adverse events will be done separately for the period 30 minutes – day 7. In addition solicited adverse events ongoing after day 7 will be presented as unsolicited AE.

For erythema and induration, recorded originally as diameters (mm), the following categorization will be used to summarize the data:

Type II: Grade 0 (< 25 mm), any (25-50 mm, 51-100 mm, >100 mm)

Body temperature will be broken down by route of measurement according to the recommendations of the Brighton collaboration and will be summarized according to the 3 schemes described below:

- by 0.5 °C increments: <36.0, 36.0 - 36.4, 36.5 - 36.9, 37.0 - 37.4, 37.5 - 37.9, 38.0 - 38.4, 38.5 - 38.9, 39.0 - 39.4, 39.5 - 39.9, ≥40.0°C
- by 1.0 °C increments: <36.0, ≥36.0-<37.0, ≥37.0-<38.0, ≥ 38.0-<39.0, ≥39.0-<40, ≥40°C
- <38.0, ≥38.0 °C

Fever, defined as a body temperature of ≥38°C irrespective of route of measurement, will be integrated to the summaries as a systemic adverse event.

The analyses will encompass summaries of the data on five levels:

1. Daily reports of subjects with solicited adverse events.
2. Time of first onset of solicited adverse events.
3. Solicited adverse events, maximum event severity by event and interval (30 min – day 7).
4. Duration of solicited adverse events, including ongoing AE after Day 7.
5. Solicited adverse events and indicators of solicited adverse events, occurrence of at least one event by category (local, systemic) and interval (30 min- Day 7).

For each of the time points or time intervals presented in the summaries, only subjects with at least one plausible observation (i.e., any non-missing values but excluding “Not done/unknown” and implausible values) for the solicited adverse events in the interval of interest will be considered. Subjects without plausible data (i.e. missing values or

reported as "Not done/unknown" and implausible values) will be removed from the denominator to prevent a downward bias (towards zero).

Level 1: Daily reports of solicited adverse event

For each of the time points only subjects with at least one plausible observation (i.e., any non-missing values but excluding "Not done/unknown" and implausible values) for the solicited adverse event in the interval of interest will be considered. Subjects without plausible data (i.e. missing values or reported as "Not done/unknown" and implausible values) will be removed from the denominator in order to prevent a downward bias (towards zero). Data collected will be summarized (frequencies and percentages of subjects) by vaccine group, solicited adverse event, vaccination number and time point.

Level 2: Time of first onset of solicited adverse events

The **time of first onset** is defined, for each subject, for each solicited adverse event, as the time point at which the respective solicited adverse event first occurred. For erythema and induration the following threshold(s) will be used ≥ 25 mm. The summary will provide the frequencies and percentages of subjects with first onset of each solicited adverse events by vaccine group and by each time point.

For each vaccination the first onset of the adverse event will be used for each subject. For any vaccination the worst adverse event across all vaccinations per time point will be used. Note, 'not done' is treated identical to 'missing'.

Level 3: Solicited adverse events, maximum event severity by event and interval

The **maximum event severity** will be defined if there is at least one plausible non-missing observation (excluding "Not done/unknown" and implausible values) within this time interval, Each subject's data will be aggregated across the time points of the interval and summarized according to the maximal severity observed for each adverse event, followed by a summary across subjects for each vaccine. Subjects without any solicited adverse events in the interval, i.e., missing values at each of the requested time points, will be removed from the denominator.

Level 4: Solicited adverse events, occurrence of at least one event by category (local, systemic) and interval.

The **occurrence of at least one solicited adverse event** is defined as "any" for a subject if he/she reports greater than "none"(≥ 25 mm for erythema and induration) for the respective event and "none" otherwise. The occurrence of at least one solicited adverse event (i.e., none versus any) will be summarized by category (i.e., local, systemic, any),

by vaccine group, by vaccination (after each vaccination and after any vaccination) and by time interval.

Medications to treat or prevent pain or fever will be summarized by frequencies and percentages of subjects reporting use of the medications by interval (30 minutes – day 7).

13.3 Unsolicited Adverse Events

All AEs occurring during the first during 28 days after each vaccination, including the day of vaccination, and all AEs leading to withdrawal, all SAEs and all AEs of special interest occurring at any time during the study will be recorded according to the protocol-specified reporting rules.

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 unsolicited adverse events will then be grouped by MedDRA preferred terms into frequency tables according to system organ class. Adverse events judged by the investigator as at least possibly related to study vaccine will be summarized by vaccine group, according to system organ class and preferred term within system organ class. When an unsolicited adverse event occurs more than once for a subject, the maximal severity and strongest relationship to the vaccine group will be counted.

Only vaccine-emergent adverse events (see section 8.7 for definition) will be analyzed, i.e., excluding those after a subject has given informed consent but before vaccination. The selection of unsolicited adverse events and the assignment to time intervals will be done by day of onset and not by days ongoing/persisting.

The summaries will be presented by period of onset and will include frequency distributions of the different adverse events:

- Onset between day 1 and study termination.

The analysis of unsolicited adverse events comprises the following categories:

- Any unsolicited adverse event.
- Possibly or probably related unsolicited adverse events.
- Unsolicited adverse events leading to death.
- Serious adverse events.
- Possibly or probably related serious adverse event.
- Unsolicited adverse events leading to premature withdrawal from study.

- Unsolicited adverse events leading to dose reduction, interruption or delay in study vaccination.
- Unsolicited adverse events leading to hospitalization.

Solicited adverse events continuing beyond day 7 will be coded by MedDRA and combined with the respective unsolicited adverse events.

13.4 Combined Solicited and Unsolicited Adverse Events

A summary of subjects with all combined solicited (regardless of their duration) and unsolicited adverse events will be provided. Solicited adverse events will be coded by MedDRA. For clintrial.gov and EudraCT posting purposes, a summary of combined solicited and unsolicited non-serious adverse events will be produced by System Organ Class and according to occurrence of each event. A further differentiation of combined adverse events according to seriousness, severity, or relationship is not performed.

13.5 Clinical Safety Laboratory Investigations

Clinical safety laboratory values, and change-from-baseline values (day x to day 1), will be summarized (mean, standard deviation, median, minimum and maximum) at each time-point of assessment, by vaccine group, for the subset of subjects in the Overall Safety Set with available laboratory data.

The frequencies of subjects with clinical laboratory values below, within, or above normal ranges will be tabulated for each clinical laboratory variable by vaccine-group and time-point of assessment (3 x 3 shift tables).

A summary of clinically significant laboratory abnormalities by time point will be provided.

Laboratory values that are outside the normal range will also be flagged in the data listings, along with corresponding normal ranges and assessment of clinical significance.

For subjects presenting at least one clinically significant value, an additional listing will be provided of all laboratory results by vaccine group, by subject, and by relevant parameter. Clinical significance assessed by the investigator will be presented.

13.6 Concomitant Medication

The frequencies and percentages of subjects reporting concomitant medications will be tabulated overall and by vaccine group. Medications (generic drug name) will be coded using the WHODRUG dictionary (see section 8.7 for definition).

14. INTERIM ANALYSIS

14.1 Interim Analysis

There are no planned interim analyses for this study.

14.1.1 Futility Analysis

Not Applicable.

15. DATA MONITORING COMMITTEES

Follow the GVGH departmental SOPs (PHV 2502) and regulatory agency guidance documents on data monitoring committees.

Refer to the study specific DSMB charter if applicable, for further details on interim analysis.

16. PEER REVIEW

Peer review of analyses should be performed in accordance with the applicable procedures for validation of SAS programs used in clinical data analysis (see BCDM-17).

The following analyses are identified in the PID as key analyses to be peer reviewed by a biostatistician independent from the study:

- Immunogenicity analysis.

The programs to be peer reviewed by a second SP will be identified in the PID.

17. LIST OF FINAL REPORT TABLES, LISTINGS AND FIGURES

For the complete list of tables, listings and figures, please refer to the Table of Contents (TOC). Note that a listing of individual efficacy/immunogenicity lab data (see "Other TOC" tab in the TOC) will be created, to allow data dissemination to the study subjects. This listing will show the following data:

- Subject ID
- Actual vaccine(s) received by the subject
- Lab results for immunogenicity safety at each visit.

This listing is mentioned in the tab "Other TOC" in the TOC and will not be part of the CSR stat package.

18. LAYOUT SPECIFICATIONS FOR TABLES, LISTINGS AND FIGURES

All TFL is to include the following header:

GVGH	Vaccine: 1790GAHB
Final Report: Study H03_04TP	Two Doses in Healthy Adults

In all tables, listings and graphs investigational 1790GAHB vaccine groups will be labeled as

Group	Description	Label
A	1790GAHB with 25 μg of GMMA total protein	**25mcg_Shi**
B	1790GAHB with 100 μg of GMMA total protein	**100mcg_Shi**
C	control group receiving one dose of Menveo as 1st injection and one dose of Boostrix as 2nd injection	**Control**

For the mock-up catalogue to be used during programming, please refer to the document stored in within the SAS Drug Development (SDD) server.

Since all TFLs will be produced using SAS, the output actually generated may slightly differ from the mock-ups presented in the study specific Mock-up catalogue.

19. REFERENCES

Nauta J. Statistics in Clinical Vaccine Trials. 2010. Heidelberg: Springer.

U.S. Department of Health and Human Services, Food and Drug Administration, CBER (2007): Guidance for Industry Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials

Clopper CJ, Pearson ES. The use of confidential or fiducial limits illustrated in the case of the binomial. Biometrika 1934; 26:404-413