GlaxoSmithKline	**Statistical Analysis Plan**
Detailed Title:	A phase IIb, randomized, placebo-controlled, single center, observer-blind, human-challenge study to evaluate the efficacy, safety and immunogenicity of 2 vaccinations with GVGH *Shigella sonnei* vaccine (1790GAHB) administered by intramuscular route in healthy non-immune adult population.
eTrack study number and Abbreviated Title	205626 (S SONNEI MONO GMMA SBVGH-003 [H03_03TP])
Scope:	All primary and secondary objectives pertaining to the above study and a subset of the tertiary objectives. The Analysis Plan for remaining tertiary objectives will be part of a separate SAP.
Date of Statistical Analysis Plan	Amendment 1 Final: 03-Sep-2019

APP 9000058193 Statistical Analysis Plan Template (Effective date: 14 April 2017)

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LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Events of Special Interest
ATC	Anatomical Therapeutic Chemical (ATC) Classification System
BMGF	Bill and Melissa Gates Foundation
CBER	FDA Center for Biologics Evaluation and Research
CHIM	Controlled Human Infection Model
CI	Confidence Interval
CTRS	Clinical Trial Registry Summary
eCRF	electronic Case Report Form
ELISA	Enzyme-linked immunosorbent assay
EoS:	End of Study
ER	Emergency Room
ES	Exposed Set
EU/ml	ELISA unit per milliliter
FAS	Full Analysis Set
Fc	Fragment crystallisable
FDA	Food and Drug Administration, United States of America
GMC	Geometric mean antibody concentration
GSK	GlaxoSmithKline
GMMA	Generalized Modules for Membrane Antigens
GMR	Geometric Mean Ratio
GMT	geometric mean titers
GVGH	GSK Vaccines Institute for Global Health
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
ICF	Informed Consent Form
IDAC	Independent Data Analysis Center
IDMC	Independent Data Monitoring Committee
IgG	Immunoglobulin G
IM	Intramuscular

LL	Lower Limit of the confidence interval
LLOQ	Lower Limit of Quantification
LPS	Lipopolysaccharide
LSAF	Life Science Analytics Framework
L-SBA	Luminescent Serum Bactericidal Assay
MedDRA	Medical Dictionary for Regulatory Activities
MSD	Moderate to Severe Diarrhea
OAg	O antigen
PBMC	Peripheral Blood Mononuclear Cells
PCD	Primary Completion Date
PD	Protocol Deviation
PPS	Per Protocol Set
qPCR	quantitative Polymerase Chain Reaction
RCDC	Reverse Cumulative Distribution Curve
RR	Risk Ratio
S. sonnei	Shigella sonnei
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SBA	Serum Bactericidal Assay
SBIR	System Built for Internet Randomization
SD	Standard Deviation
sIgA	Secretory Immunoglobulin A
TFL	Tables Figures and Listings
TOC	Table of Content
UL	Upper Limit of the confidence interval
Vacc	Vaccination
VE	Vaccine Efficacy

1. DOCUMENT HISTORY

Date	Description	Protocol Version
09-AUG-2018	Final Version	22-JUN-2018 Amendment 2 Final
03-SEP-2019	Amendment 1	23-JUL-2019 Amendment 6 Final

2. STUDY DESIGN

This is a randomized, placebo-controlled, single center, observer-blind, phase 2b human challenge trial. The study includes a screening visit (performed between study Days -45 to -1), 2 clinical visits with vaccination (performed at study Day 1 and Day 29), 2 clinical visits 7 days after each vaccination (performed at study Day 8 and Day 36), 1 clinical visit with *S. sonnei* 53G Challenge administration during an 8-day inpatient stay 28 days after second vaccination (performed at study Day 57), and 3 visits after challenge, 28 days (study Day 85), 56 days (study Day 113) and 6 months (study Day 237) after challenge.

This study will evaluate the efficacy, safety and immunogenicity of the 1790GAHB vaccine administered via intramuscular (IM) route to adults (18-50 years of age at enrolment). The objectives will be assessed through the conduct of the study in two phases: a vaccination phase with 1790GAHB vaccine or placebo followed by a challenge phase with *S. sonnei* strain 53G.

A total of 72 subjects will be divided in 4 cohorts of 18 subjects each. The vaccination and challenge will be done in overlapping cohorts and randomization will ensure there is the same number of vaccinees and controls in each cohort. However, if for any reason, a cohort did not reach the planned 18 subjects, additional subjects can be added to the following cohorts. The maximum number of subjects must not be more than 20 (maximum number of beds) in each cohort.

- **Experimental design:** Phase IIB, observer-blind, randomized, placebo control, mono-centric study with two parallel groups (*S. sonnei* group and placebo group).
- **Duration of the study**: Each subject will be followed up for approximately 6 months after challenge with the pathogenic *S. sonnei* strain 53G, with a total study duration of approximately 32 weeks (i.e., 8 months) for each study subject.
  - Epoch 001: Starting at Screening Visit (Day -45 to Day -1) and ending before Visit 1 (Day 1).
  - Epoch 002: Starting the day of randomization and first vaccination (Visit 1) and ending before the receipt of the challenge agent, 28 days after second vaccination (Visit 5).
  - Epoch 003: Starting with the receipt of the challenge agent at 28 days after second vaccination (Visit 5) and ending 6 months after challenge (Visit 8).

- **Primary completion date (PCD):** Visit 8 (6 months after challenge).

  Refer to Glossary Section of the protocol for the definition of PCD.

- **End of Study (EoS):** Last testing results released of samples collected for primary and secondary objectives at Visit 8.

  Refer to Glossary Section of the protocol for the definition of EoS.

- **Study groups:**

Table 1 Study groups and epochs foreseen in the study

Study groups	Number of subjects	Age (Min - Max)	Epochs		
			Epoch 001	Epoch 002	Epoch 003
			Screening	1st and 2nd vaccination	Challenge
S. sonnei	36	18 years – 50 years	x	x	x
Placebo	36	18 years – 50 years	x	x	x

Table 2 Study groups and treatment foreseen in the study

Treatment name	Vaccine/ placebo/ challenge agent name	Study Groups	
		S. sonnei	**Placebo**
S. sonnei	1790GAHB	x	
Placebo	GAHB-Placebo		x
Challenge	*S. sonnei* 53G strain	x	x

- **Control:** Placebo control with GAHB-Placebo with the same composition as the vaccine except the active ingredient. GAHB-Placebo will also be used as diluent for bed-side mixing of the vaccine.
- **Vaccination schedule:** Subjects will receive 2 doses of either the study vaccine or placebo 28 days apart. At 28 days after the second dose, all subjects will receive the challenge dose.
- **Treatment allocation:** Following the screening period before the first vaccination (Day -45 to Day -1), subjects will be randomized in a 1:1 ratio to receive either the study vaccine or the placebo.
- **Blinding:** The study will be observer-blind.

Table 3 Blinding of study epochs

Study Epochs	Blinding
Screening (Epoch 001)	N/A
1st and 2nd vaccination (Epoch 002)	observer-blind
Challenge (Epoch 003)	observer-blind

An overview of the study design is given in Figure 1 and a list of study procedures is reported in Table 4. For further details please refer to Section 3 of the protocol.

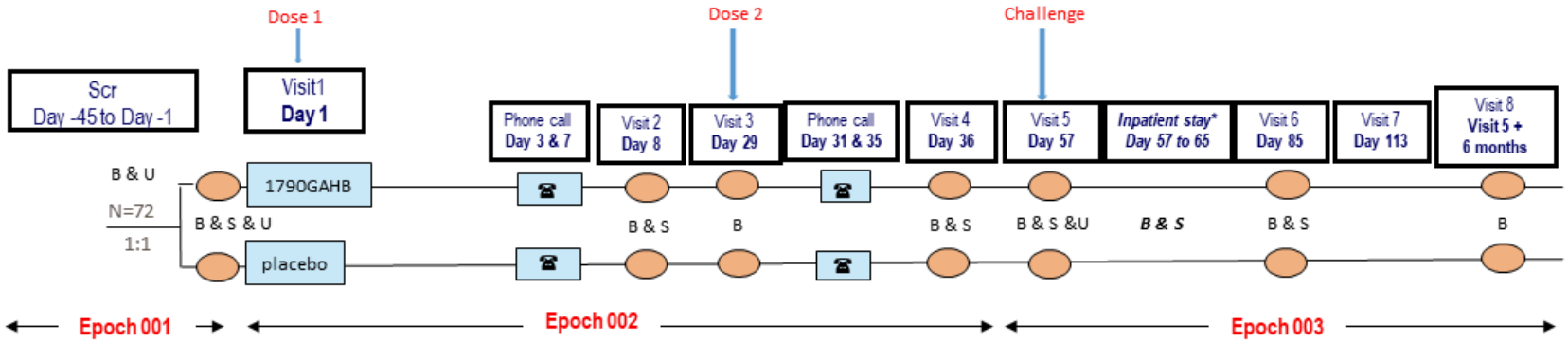
Randomization

After obtaining the signed and dated ICF from the subject and having checked the eligibility of the subject, the site staff in charge of the vaccine administration will access GSK Biological's Internet Randomization System (SBIR). Upon providing the subject identification number, the randomization system will determine the study group and will provide the treatment number to be used for each dose.

The number of each administered treatment must be recorded in the eCRF (electronic Case Report Form) on the Vaccine Administration screen.

The target will be to enroll approximately 72 eligible subjects (36 per treatment group) who will be randomly assigned to two study groups in a (1: 1) ratio. The randomization algorithm will use a minimization procedure accounting for study. The block size used for the material randomization list is 6.

Figure 1 Study Design



Refer to Section 3.2 from Protocol for details of procedures during the inpatient stay (Amended: 23 July 2019)
Scr: Screening
N: Number of subjects
B: Blood sampling
S: Stool sampling
U: Urine sampling

Table 4 List of study procedures

Epoch	Screening	Vaccination						Challenge				
Type of contact	Screening visit	Visit 1	Phone Call	Visit 2	Visit 3	Phone call	Visit 4	Visit 5	Inpatient stay	Visit 6	Visit 7	Visit 8
Timepoints (days)	Day -45 to Day -1	Day 1	Day 3 and Day 7	Day 8	Day 29	Day 31 and Day 35	Day 36	Day 57	Day 57 to Day 65 [8]	Day 85	Day 113	Day 237 (Month 8)
Sampling timepoints	Screening	Pre-vacc 1		Post-vacc 1	Pre-vacc 2		Post-vacc 2	Pre-challenge[9]	Inpatient stay	Post-challenge		
Informed consent	●											
Check inclusion/exclusion criteria	●				●			●				
Collect demographic data	●											
Measure/record height and weight	●											
Medical history	●											
History directed physical examination				O			O		O	O	O	O
Physical examination	●	●			●			●				
Urine pregnancy test [1]	●	●			●			●				●
Check contraindications to vaccination	O	O			O							
Pre-vaccination/placebo/challenge agent administration body temperature		●			●			●				
Vaccine/placebo/challenge agent administration												
Study group and treatment number allocation		●										
Recording of administered treatment number		●			●							
Vaccine/placebo administration		●			●							
Challenge with *S. sonnei* 53G								●				
Laboratory assays												
Blood sampling for antibody response (~20 mL)		● [2, 3]		●	● [2]		●	● [2, 3]	● [4]	●		
Blood sampling for PBMC isolation (α4β7 plasmablasts response and transcriptomics) (~***50*** mL)		●		●			●		● [4]			
Blood sampling for hematology (~5 mL) [5]	●			●	●		●	●				●
Blood sampling for biochemical analysis (~5 mL)	●											

Epoch	Screening	Vaccination						Challenge				
Type of contact	**Screening visit**	**Visit 1**	**Phone Call**	**Visit 2**	**Visit 3**	**Phone call**	**Visit 4**	**Visit 5**	**Inpatient stay**	**Visit 6**	**Visit 7**	**Visit 8**
Timepoints (days)	**Day -45 to Day -1**	**Day 1**	**Day 3 and Day 7**	**Day 8**	**Day 29**	**Day 31 and Day 35**	**Day 36**	**Day 57**	**Day 57 to Day 65** [8]	**Day 85**	**Day 113**	**Day 237 (Month 8)**
Sampling timepoints	**Screening**	**Pre-vacc 1**		**Post-vacc 1**	**Pre-vacc 2**		**Post-vacc 2**	**Pre-challenge**[9]	**Inpatient stay**	**Post-challenge**		
Serology for virology: HIV, HBV, HCV (~10 mL)	●											
Blood test for HLA-B27 and local anti *S. sonnei* LPS IgG (Screening-ELISA) (~15 mL)	●											
Urine sampling for urinalysis [6]	●							●				
Stool sample for sIgA		●		●			●		● [4]			
Stool sample for microbiome testing [7]		●						●				
Stool assessment: weight, consistency, blood and *S. sonnei* by culture and qPCR									●			
Safety assessment												
Record any concomitant medications/vaccinations	●	●		●	●		●	●	●	●	●	
Distribution of diary cards		O			O							
Return of diary cards				●			●					
Diary card transcription by investigator				●			●					
Recording of solicited adverse events (1–7 days post-vaccination)				●			●					
Recording of non-serious adverse events within 28 days post-vaccination				●	●		●	●				
Recording of AESI, SAEs and pregnancies	●	●		●	●		●	●	●	●	●	●
Recording of SAEs related to study participation, or to a concurrent GSK medication/vaccine	●	●		●	●		●	●	●	●	●	●
Study Conclusion												●

V: Visit, **D**: Day, **Vacc**: Vaccination; **HIV**: Human Immunodeficiency Virus, **HCV**: Hepatitis C Virus, **HBV**: Hepatitis B Virus, **ELISA**: Enzyme-linked immunosorbent assay, **AESI**: Adverse Events of Specific Interest, **SAEs**: Serious Adverse Events.

● is used to indicate a study procedure that requires documentation in the individual eCRF.

O is used to indicate a study procedure that does not require documentation in the individual eCRF.
[1] Female subjects will be randomized and study vaccine/placebo or challenge agent may only be administered if the pregnancy test is negative.
[2] Serum bactericidal assay will be performed at these visits in addition to the *S. sonnei* LPS IgG antibody response.
[3] Fc glycosylation test on leftover serum samples will be performed at these visits in addition to the *S. sonnei* LPS IgG antibody response and serum bactericidal assay.
[4] Sampling to be done 7 days after challenge agent administration (i.e., Day 64).
[5] Additionally, hematology is repeated weekly until resolution if neutropenia occurs. On the day of vaccination/challenge, sampling will be done before vaccination/challenge
[6] Urine dipstick to be done for all subjects and, in case of abnormal results, a urine culture is to be performed (urinalysis).
[7] Before vaccination/challenge (Day 1/Day 57 sample can be collected by the subject at most a week before and stored in a freezer).
[8] Sampling to be done daily during inpatient stay and if subject is still shedding 7 days post-challenge administration (Day 64). However, all subjects even if not shedding anymore will remain in the inpatient stay until 7 days post-challenge and will be discharged on Day 65.
[9] Samples collection can be performed between Day 56 and Day 57.

Whenever possible, the investigator should arrange study visits within the interval described in Table 5.

Table 5 Intervals between study visits

Interval	Optimal length of interval	Allowed interval
Screening visit →.Visit 1	-7 days	-45 days – -1 days
Visit 1→ Phone Call	3 days	2 days - 6 days
Visit 1 →.Visit 2	7 days	7 days - 10 days
Visit 1 → Visit 3	28 days	26 days - 33 days
Visit 3 → Phone call	3 days	2 days - 6 days
Visit 3 → Visit 4	7 days	7 days - 10 days
Visit 3 → Visit 5	28 days	26 days - 33 days
Visit 5 → Inpatient stay	9 days	9 days - as needed
Visit 5 → Visit 5 post-challenge blood draw	7 days	7 days - 10 days
Visit 5 → Visit 6	28 days	28 days - 35 days
Visit 5 → Visit 7	56 days	49 days - 63 days
Visit 5 → Visit 8	180 days	166 days - 194 days

3. OBJECTIVES

3.1. Primary objective

Efficacy

- To demonstrate the efficacy of two vaccinations with 25 µg of 1790GAHB vaccine in healthy adults compared to placebo in reducing shigellosis, fulfilling the primary protocol case definition (reported in Section 4.1), after challenge with S. sonnei strain 53G.

Criterion:

- Vaccine efficacy (VE) of 1790GAHB vaccine will be shown if the lower limit (LL) of the 90% confidence interval (CI) of VE will be above zero.

Refer to Section 4.1 below for the protocol case definition of shigellosis and to Section 4.2 for the definition of the primary endpoint.

3.2. Secondary objectives

Efficacy

- To determine the efficacy of the 1790GAHB vaccine compared to placebo against:
  - Shigellosis, as per definition requested by the Controlled Human Infection Model (CHIM) expert working group (see Section 6.3.3).
  - Shigellosis, as defined by: severe diarrhea OR moderate diarrhea with fever or with one or more moderate constitutional/enteric symptoms OR dysentery [[≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [≥1 at least mild constitutional/enteric symptom]].
  - More severe shigellosis, as defined by: severe or moderate diarrhea with fever or with one or more at least severe constitutional/enteric symptoms OR dysentery [[≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND fever OR ≥1 at least severe constitutional/enteric symptom]].
  - Shedding of *S. sonnei* strain 53G
  - Severe diarrhea.
  - More severe diarrhea.
  - Dysentery.
  - Weight of all grade 3-5 stools.
  - The total number of grade 3-5 stools.

- Confirmed *S. sonnei* 53G shedding AND [moderate or severe diarrhea OR dysentery OR presence of fever ≥ 38.5°C OR presence of one or more severe intestinal symptoms (abdominal pain, cramping, nausea, vomiting, gas, and anorexia)].
- Disease not fulfilling the protocol primary case definition for shigellosis associated or not with mild to moderate symptoms including: passing loose stool (not meeting the protocol definition of moderate or severe diarrhea), abdominal pain, abdominal cramps, gas, anorexia, nausea, headache, myalgia, malaise, arthralgia, fever, vomiting and IV fluid administration.
- Time to onset of shigellosis after challenge

Safety

- To assess the safety and reactogenicity of the 1790GAHB vaccine in terms of solicited symptoms, unsolicited symptoms, serious adverse events (SAEs), adverse events of special interest (AESI) and laboratory parameters.
- To assess safety after challenge in terms of SAEs, unsolicited symptoms, AESI and laboratory parameters.

Immunogenicity

- To evaluate the IgG ELISA (Enzyme-linked immunosorbent assay) immunogenicity profile of the 1790GAHB vaccine at 7, and 28 days after the first and second vaccination (IgG ELISA coated with O antigen (OAg) containing Lipopolysaccharide (LPS)).
- To evaluate the immunogenicity profile of the 1790GAHB vaccine at pre-challenge and at 7 and 28 days after challenge (IgG ELISA coated with OAg containing LPS).
- To assess seroresponse for anti-*S. sonnei* LPS at 7 and 28 days after first and second vaccination.
- To assess post vaccination concentration ≥ 121 EU/ml for anti-LPS *S. sonnei* at 7 and 28 days after first and second vaccination
- To assess post challenge concentration ≥ 121 EU/ml for anti-LPS *S. sonnei* at 7 and 28 days after challenge

Refer to Section 4.3 for the definition of the secondary endpoints.

3.3. Tertiary objectives

The following tertiary objectives will be analyzed as part of this SAP:

- Evaluate serum bactericidal activity against *S. sonnei* at Day 1 and 28 days after first and second vaccination.
- Evaluate correlation between serum anti-*S. sonnei* LPS IgG level and serum bactericidal activity titer.

The following and remaining tertiary objectives will complement assessment of the vaccine immunogenicity profile and will be part of a subsequent SAP:

- Evaluate *S. sonnei* specific Secretory Immunoglobulin A (sIgA) in stool at Day 1 and 7 days after first and second vaccination and 7 days after challenge (Day 64).
- Evaluate *S. Sonnei* LPS IgG-specific α4β7+/- plasmablast at Day 1 and 7 days after first and second vaccination and 7 days after challenge (Day 64).
- Evaluate correlation between serum anti-*S. sonnei* LPS IgG level, serum bactericidal activity titer, shigellosis, shedding, mild, moderate, severe or more severe diarrhea, and dysentery after challenge.
- Evaluate glycosylation of fragment crystallisable (Fc) portion of IgG antibodies at Day 1 and 28 days after second vaccination and association with clinical protection and/or serological endpoints.
- Evaluate gene expression signatures at Day 1 and 7 days after first and second vaccination and 7 days after challenge (Day 64).
- Investigate gut microbiome at Day 1 and 28 days after second vaccination and its potential association with protection and immune response.

These last two tertiary objectives involve pharmacogenomics testing. Refer to the Glossary of the protocol for the definition of Pharmacogenomics.

Refer to Section 4.4 for the definition of the tertiary endpoints.

4. ENDPOINTS

4.1. Case Definitions

Stools

Stools will be graded as follows:

- Grade 1: firm formed;
- Grade 2: soft formed;
- Grade 3: viscous opaque liquid or semi-liquid which assumes the shape of the bowl;
- Grade 4: watery opaque liquid;
- Grade 5: clear watery or mucoid liquid.

Diarrhea

For the purpose of this study, diarrhea as used in the primary endpoint is defined as:

- Moderate diarrhea: 4 to 5 loose or watery (Grade 3 to 5) stools or 400 to 800 grams of Grade 3 to 5 stools within 24 hours.

- Severe diarrhea: 6 or more loose or watery (Grade 3 to 5) stools or > 800 grams of Grades 3 to 5 stools within 24 hours or required medical intervention.
- More severe diarrhea: ≥ 10 loose or watery (Grade 3 to 5) stools or ≥ 1000 grams of Grade 3 to 5 stools within 24 hours.
- Note: Diarrhea severity classes are not mutually exclusive, as more severe diarrhea is a subset of severe diarrhea.
- In case of severe diarrhea, medical intervention is defined as intravenous (IV) fluids administration or anticipation of antibiotic treatment before the 5th day after challenge.

Dysentery

For the purpose of the endpoint "Rate of shigellosis as defined by the CHIM expert working group occurring within a period starting with challenge visit and lasting until the end of the inpatient stay, in all subjects" (a), see Section 4.3), dysentery is defined as follows:

- At least 2 loose stools with gross blood (hemoccult positive) in 24 hours AND [oral temperature ≥38.0°C OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours]. In this case constitutional/enteric symptom are the following: nausea, abdominal pain, abdominal cramping, myalgia, arthralgia, malaise.

For the purpose of the endpoint "Rate of shigellosis, as defined by: severe diarrhea OR moderate diarrhea with fever or with one or more moderate constitutional/enteric symptoms OR dysentery" (b), see Section 4.3), dysentery is defined as follows:

- At least 2 loose stools with gross blood (hemoccult positive) in 24 hours AND [≥1 reportable constitutional/enteric symptom]. In this case constitutional/enteric symptom are the following: headache, fatigue, arthralgia, malaise, myalgia, chills, nausea, abdominal cramping, abdominal pain, gas, anorexia, vomiting.

For the purpose of the endpoint "Rate of more severe shigellosis as defined by: severe or moderate diarrhea with fever or with one or more severe constitutional/enteric symptom OR dysentery [[≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [oral temperature ≥38.0°C OR ≥1 severe constitutional/enteric symptom]]." (c), see Section 4.3), dysentery is defined as follows:

- At least 2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [oral temperature ≥38.0°C OR ≥1 severe constitutional/enteric symptom]. In this case constitutional/enteric symptom are the following: headache, fatigue, arthralgia, malaise, myalgia, chills, nausea, abdominal cramping, abdominal pain, gas, anorexia, vomiting.

For the purpose of secondary efficacy objective g) and k) (see Section 4.3), dysentery is defined as:

- A Grade 3, 4, or 5 stool with gross blood on at least 2 occasions within 24 hours AND presence of constitutional/enteric symptoms (i.e. fever, headache, fatigue, arthralgia, malaise, myalgia, chills, nausea, abdominal cramping, abdominal pain, gas, anorexia, vomiting).

Shigellosis

For the challenge phase of the study, the protocol primary case definition for shigellosis is:

- Shedding of *S.sonnei 53G* accompanied by moderate or severe diarrhea OR shedding with an ***oral temperature ≥ 38.5°C***.

4.2. Primary endpoint

Primary Efficacy endpoint

Vaccine efficacy (VE) will be evaluated in the 8-day post-challenge inpatient period after visit 5 (i.e. from challenge to discharge) by:

- Attack Rate of shigellosis (fulfilling the protocol case definition) occurring within a period starting with the challenge visit and lasting up to the end of the inpatient stay, in all subjects. The attack rate of shigellosis is expressed as proportion of subjects per group with at least one episode of shigellosis after challenge.

4.3. Secondary endpoints

Secondary Efficacy endpoints

All the secondary endpoints will be assessed in the 8-day post-challenge inpatient period after visit 5 (i.e. from challenge to discharge).

All subjects receiving the 1790GAHB vaccine vs. placebo will be compared according to the following endpoints:

a) Attack rate of shigellosis, as defined by CHIM expert working group (see Section 6.3.3)

b) Attack rate of shigellosis, as defined by any one of the three following possible endpoints:

- Severe diarrhea defined as [≥6 loose stools in 24 hours OR >800 grams loose stools in 24 hours]

- Moderate diarrhea defined as [4-5 loose stools in 24 hours OR 400-800 grams loose stools in 24 hours] AND [oral temperature ≥38.0^{o}C OR ≥1 at least moderate constitutional/enteric symptom].

- Dysentery defined as [≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [≥1 at least mild constitutional/enteric symptom].

c) Attack rate of More Severe Shigellosis, as defined by any one of the two following possible endpoints:

- Moderate OR severe diarrhea [≥4 loose stools in 24 hours OR ≥400 grams loose stools in 24 hours] AND [oral temperature ≥38.0ºC OR ≥1 at least severe constitutional/enteric symptom].

- Dysentery defined as [≥2 loose stools with gross blood (hemoccult positive) in 24 hours] AND [oral temperature ≥38.0ºC OR ≥1 at least severe constitutional/enteric symptom].

d) Proportion of subjects with shedding of *S. sonnei* strain 53G.

Shedding of *S. sonnei* strain 53G is defined as positivity of at least one stool sample either by culture or quantitative Polymerase Chain Reaction (qPCR).

e) Proportion of subjects with at least one episode of severe diarrhea.

f) Proportion of subjects with at least one episode of more severe diarrhea.

g) Proportion of subjects with at least one episode of dysentery (as defined in Section 4.1).

h) Mean (arithmetic or geometric, according to distribution of the data) weight of grade 3-5 stools per subject, for all subjects and for subjects with at least one grade 3-5 stool (i.e. grade 3-5 stool).

i) Mean weight per subject of all grade 3-5 stools accumulated from challenge to discharge

j) Mean number of grade 3-5 stools per subject from challenge to discharge.

k) Proportion of subjects with confirmed S. sonnei 53G shedding AND [moderate or severe diarrhea, OR dysentery (as defined in Section 4.1), OR presence of fever (oral temperature ≥ 38.5°C), OR presence of at least one intestinal symptom (abdominal pain, abdominal cramping, gas, nausea, vomiting, anorexia) graded as severe].

l) Disease not fulfilling the protocol case definition for shigellosis associated or not with mild to moderate symptoms including:

1. Proportion of subjects with at least one stool (not meeting the protocol definition of moderate or severe or more severe diarrhea),
2. Proportion of subjects with at least one episode of abdominal pain,
3. Proportion of subjects with at least one episode of abdominal cramps,
4. Proportion of subjects with at least one episode of gas,
5. Proportion of subjects with at least one episode of anorexia,
6. Proportion of subjects with at least one episode of nausea,
7. Proportion of subjects with at least one episode of headache,
8. Proportion of subjects with at least one episode of myalgia,
9. Proportion of subjects with at least one episode of malaise,

10. Proportion of subjects with at least one episode of arthralgia,
11. Proportion of subjects with at least one episode of fever (oral temperature ≥ 38.5°C),
12. Proportion of subjects with at least one episode of vomiting, and
13. Proportion of subjects with at least one IV fluid administration.

m) Time to onset of Shigellosis during the post-challenge period

All endpoints are measured from challenge to discharge.

Note that in the primary and secondary efficacy endpoints #k and #l.11, fever is defined as oral temperature ≥ 38.5°C, in agreement with challenge model set up at Cincinnati. However, fever in other endpoints and as solicited event is defined as a body temperature of ≥38°C irrespective of route of measurement, in agreement with latest GSK standards and Brighton collaboration rules (see Section 6.5.2.2).

For derivation of the primary and secondary endpoints refer to Section 12.2.7.

Secondary Safety endpoints

The measures of safety will include:

- Number of subjects with solicited local and systemic reactions during 7 days following each vaccination. Solicited local reactions include injection site erythema, injection site induration and injection site pain; solicited systemic reactions include headache, arthralgia, chills, fatigue, malaise, myalgia, and fever (as measured orally).
- Number of subjects with reported unsolicited adverse events (of any nature and severity) during 28 days following vaccination and challenge, i.e. up to second vaccination visit (when evaluating after first vaccination) whichever comes first, and up to challenge visit (when evaluating after second vaccination) whichever comes first, and up to 28th day after challenge.
- Number of subjects with reported SAEs throughout the study duration.
- Number of subjects with reported AESI (i.e., symptomatic neutropenia) throughout the study duration.
- Number of subjects with deviations from normal values (i.e. reference range and/or clinically significant value, according to local ranges) of hematological tests at 7 days after first and second vaccination (Visit 2 and Visit 4) and at last study visit (Visit 8).

Secondary Immunogenicity endpoints

The measures of immunogenicity, against the LPS of *S. sonnei*, will include:

- IgG geometric mean concentrations (GMCs) pre-vaccination (Day 1), 7 days after first vaccination (Day 8), 28 days after first vaccination (Day 29), 7 days after second vaccination (Day 36), and 28 days after second vaccination (Day 57) overall and by antibody concentration at baseline (i.e., above vs. below ELISA Lower Limit

of Quantification, LLOQ, 22 EU/ml), as determined by anti-*S. sonnei* LPS IgG ELISA and applicable within-subject geometric mean ratios (GMRs) between post vaccination and baseline samples (D8/D1, D29/D1, D36/D1 and D57/D1).

- IgG GMC pre-challenge (Day 57), 7 and 28 days after challenge (Day 64 and Day 85) as determined by anti-*S. sonnei* LPS IgG ELISA and applicable within-subject geometric mean ratios (GMRs) between post-challenge and pre-challenge samples (D64/D57, and D85/D57).
- Number and percentage of seroresponders for anti-*S. sonnei* LPS at 7 and 28 days after first and second vaccination. Seroresponse is aimed to define a significant increase in anti *S. sonnei* LPS IgG concentration in post-vaccination samples and relies on the definition already used in a previous phase II study in Kenyan population. See Section 6.4.2 for definition of seroresponders.
- Number and percentage of subjects with titers post vaccination concentration ≥ 121 EU/ml for anti-LPS *S. sonnei* at 7 and 28 days after first and second vaccination
- Number and percentage of subjects with titers post challenge concentration ≥ 121 EU/ml for anti-LPS *S. sonnei* at 7 and 28 days after challenge

4.4. Tertiary endpoints

Below are reported the tertiary immunogenicity endpoints in scope of this SAP:

- Luminescent Serum Bactericidal Assay (L-SBA) geometric mean titers (GMTs) pre-vaccination (Day 1), 28 days after first vaccination (Day 29), and 28 days after second vaccination (Day 57) overall and by antibody concentration at baseline (i.e., above vs. below L-SBA LLOQ), as determined by L-SBA and applicable within-subject geometric mean ratios (GMRs) between post vaccination and baseline samples (D29/D1 and D57/D1).
- Correlation between bactericidal antibodies titers (as measured by L-SBA titres) and anti-LPS S. sonnei serum IgG antibody concentration (as measured by ELISA) will be assessed by Pearson correlation coefficient, calculated on the log10 transformed values for all the sample pooled together. Correlation will be also assessed by the treatment groups (shigella and placebo) and antibodies at baseline above and below LLOQ for L-SBA and ELISA. The correlation coefficients will be graphically supplemented by scatter plots

5. ANALYSIS SETS

5.1. Definition

5.1.1. All Enrolled Set

All screened subjects who provide informed consent, and provide demographic and/or baseline screening assessments and received a Subject ID, regardless of the subject's randomization and treatment status in the study.

5.1.2. All Exposed Set

All subjects in the enrolled set who receive a study vaccination. Please note that throughout Section 5, vaccine/study vaccination refer to study vaccine or placebo.

5.1.3. Safety Set

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

All subjects in the All Exposed Set with any valid data on solicited AEs.

5.1.3.2. Unsolicited Safety Set (unsolicited adverse events)

All subjects in the Exposed Set that do not withdraw early and are lost to follow-up on a vaccination visit.

5.1.3.3. Overall Safety Set

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

5.1.4. Full Analysis Set (FAS) for Efficacy/Immunogenicity

5.1.4.1. Full Analysis Set for Efficacy

All subjects in the All Enrolled Set who are randomized, receive a study vaccination and provide efficacy data.

5.1.4.2. Full Analysis Set for Immunogenicity

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

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).

5.1.5. Per-Protocol Set (PPS) for Efficacy/Immunogenicity

All subjects in the FAS for Efficacy/Immunogenicity who:

- Correctly receive the vaccines (i.e., receive the vaccine to which the subjects is randomized and at the scheduled timepoints).
- Have no protocol deviations leading to exclusion as defined prior to unblinding / analysis.

- Are not excluded due to other reasons defined prior to unblinding or analysis.

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.

The primary analysis will be based on the PPS for efficacy and immunogenicity. However, selected analyses will be performed on both FAS and PPS sets (more details in Section 6).

PPS for Immunogenicity will be furtherly categorized in PPS Post-Vaccination where Visit 1, 2, 3, 4, and 5 are analyzed, and in PPS Post-Challenge where Visit 5, Visit at the end of inpatient stay, and Visit 6 are analyzed.

5.2. Criteria for eliminating data from Analysis Sets

Elimination codes are used to identify subjects to be eliminated from analysis. Detail is provided below for each set. Consolidated tables are also available in Section 13.

5.2.1. Elimination from Exposed Set (ES)

Code 1030 (Study vaccine not administered at all) and code 900 (invalid informed consent or fraud data) will be used for identifying subjects eliminated from ES.

5.2.2. Safety Set

Code 1150 (Subject did not provide any post-vaccination/post-challenge unsolicited safety data) will be used for identifying subjects eliminated from the Unsolicited Safety Set.

Code 1160 (Subject did not provide any post-vaccination solicited safety data) will be used for identifying subjects eliminated from the Solicited Safety Set.

Subjects with codes 1155 will be eliminated from the Overall Safety Set.

5.2.3. Elimination from Full Analysis Set (FAS) for Efficacy

5.2.3.1. Excluded subjects

A subject will be excluded from the FAS for Efficacy analysis under the following conditions

Code	Condition under which the code is used
900	Invalid informed consent or fraud data Signed informed consent not available on site Informed consent not signed and/or dated by subject Informed consent not signed prior to any study procedure Fraudulent Data
1030	Study vaccine not administered for both doses
3000.a	Did not undergo challenge
3000.c	Missed or incomplete assessment (after challenge)

5.2.4. Elimination from Per-protocol analysis Set (PPS) for Efficacy

5.2.4.1. Excluded subjects

A subject will be excluded from the PPS analysis for Efficacy under the following conditions

Code	Condition under which the code is used
900	Invalid informed consent or fraud data: Signed informed consent not available on site Informed consent not signed and/or dated by subject Informed consent not signed prior to any study procedure Fraudulent Data
1030	Study vaccine not administered for both doses
1040	Administration of concomitant vaccine(s) forbidden in the protocol*
1050	Randomization failure
1060	Randomization code was broken
1070	Vaccination not according to protocol: Incomplete treatment course

Code	Condition under which the code is used
	Route of administration is wrong or unknown Treatment administered is correct but is not compatible with treatment regimen associated to treatment schedule Wrong replacement or study treatment administered Study treatment not prepared as per protocol (e.g. reconstitution) Site of injection for vaccine
1500	Incorrect Volume was given
1500	Study treatment not available at site for administration
1500	Study treatment administered while contraindication
1500	Pregnancy
1080	Vaccine temperature deviation
1090	Expired vaccine administered
2010	Protocol violation (inclusion/exclusion criteria)
2040	Administration of any medication forbidden by the protocol*
2050	medical condition forbidden
2060	infection related to the vaccine
2070	infection not related to the vaccine
2080	Subjects did not comply with vaccination schedule
2130	Subject not planned to be sampled for their all sampling visits: Testing performed on samples not aligned with ICF Collection of multiple samples when not required Subjects bled but not supposed to be bled Sample discard/destruction not completed as per protocol/SPM/ICF Sample destroyed and should not have been destroyed
3000.a	Did not undergo challenge
3000.b	Challenge administered out of the window
3000.c	Missed or incomplete assessment (after challenge)
3000.d	Challenge impacted by temperature excursion
3000.e	Challenge not prepared as per protocol (e.g. reconstitution)
3000.f	Challenge administered while contraindication
3000.g	Subject left the inpatient stay during day 5 of inpatient stay or earlier

*See protocol Section 7.6.2 for specific details.

5.2.5. Elimination from Full Analysis Set (FAS) for Immunogenicity

5.2.5.1. Excluded subjects

A subject will be excluded from the FAS for Immunogenicity analysis under the following conditions

Code	Condition under which the code is used
900	Invalid informed consent or fraud data Signed informed consent not available on site Informed consent not signed and/or dated by subject Informed consent not signed prior to any study procedure Fraudulent Data
1030	Study vaccine not administered at all
2100	Serological results not available post-vaccination: Missed assessment (subject early terminated prior to the visit) Missed assessment (subject continues participation in the study) Low volume (not sufficient to perform testing) No samples collected for subject (and should have been collected) Mislabeling (sample not tested)
2120	Obvious incoherence or abnormality or error in data (for Antibody determination): Temperature deviations from range defined in protocol and/or SPM – refrigerator Temperature deviation from range defined in protocol and/or SPM - -20Freezer Temperature deviation from range defined in protocol and/or SPM - -45 freezer Temperature deviation from range defined in protocol and/or SPM - -80 freezer Central/internal/external lab deviations Mislabeling (sample tested)

*See protocol Section 5.3.

5.2.6. Elimination from Per-protocol analysis Set (PPS) for Immunogenicity

5.2.6.1. Excluded subjects

A subject will be excluded from the PPS analysis for Immunogenicity under the following conditions

Code	Condition under which the code is used
900	Invalid informed consent or fraud data Signed informed consent not available on site Informed consent not signed and/or dated by subject Informed consent not signed prior to any study procedure Fraudulent Data
1030	Study vaccine not administered
1040	Administration of concomitant vaccine(s) forbidden in the protocol*
1050	Randomization failure
1060	Randomization code was broken
1070	Vaccination not according to protocol: Incomplete treatment course Route of administration is wrong or unknown Treatment administered is correct but is not compatible with treatment regimen associated to treatment schedule Wrong replacement or study treatment administered Study treatment not prepared as per protocol (e.g. reconstitution) Site of injection for vaccine
1500	Incorrect Volume was given
1500	Study treatment not available at site for administration
1500	Study treatment administered while contraindication
1500	Pregnancy
1080	Vaccine temperature deviation
1090	Expired vaccine administered
2010	Protocol violation (inclusion/exclusion criteria)**
2040	Administration of any medication forbidden by the protocol*

Code	Condition under which the code is used
2050	medical condition forbidden
2060	infection related to the vaccine
2070	infection not related to the vaccine
2080	Subjects did not comply with vaccination schedule
2090	Subjects did not comply with blood sample schedule
2100	Serological results not available post-vaccination: Missed assessment (subject early terminated prior to the visit) Missed assessment (subject continues participation in the study) Low volume (not sufficient to perform testing) No samples collected for subject (and should have been collected) mislabeling (sample not tested)
2120	Obvious incoherence or abnormality or error in data (for Antibody determination): Temperature deviations from range defined in protocol and/or SPM – refrigerator Temperature deviation from range defined in protocol and/or SPM - -20Freezer Temperature deviation from range defined in protocol and/or SPM - -45 freezer Temperature deviation from range defined in protocol and/or SPM - -80 freezer Central/internal/external lab deviations Mislabeling (sample tested)
2130	Subject not planned to be sampled: Testing performed on samples not aligned with ICF Collection of multiple samples when not required Subjects bled but not supposed to be bled Sample discard/destruction not completed as per protocol/SPM/ICF Sample destroyed and should not have been destroyed

*See protocol Section 7.6.2 for specific details.

**See protocol Section 5.3.

5.3. Important protocol deviation not leading to elimination from per-protocol analysis set

Important protocol deviations not leading to elimination from PPS and FAS will be defined in the Protocol Deviation Management Plan.

6. STATISTICAL ANALYSES

Note that standard data derivation rule and stat methods are described in Section 12 Annex 1 and will not be repeated below.

6.1. Demography

6.1.1. Analysis of demographics/baseline characteristics planned in the protocol

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

Distributions of subjects by gender and geographic ancestry will be summarized for the Exposed Set.

6.1.2. Additional considerations

Demography data will be tabulated also for the Efficacy Per Protocol Set, Efficacy Full Analysis Set, Immunogenicity Per Protocol Post-Vaccination Set, Immunogenicity Per Protocol Post-Challenge Set, and All Enrolled Set.

6.2. Exposure

6.2.1. Analysis of exposure planned in the protocol

Not applicable.

6.2.2. Additional considerations

The frequencies and percentages of subjects with vaccination will be summarized overall and by vaccine/placebo group. Data will be tabulated for the All Exposed Set.

6.3. Efficacy/Effectiveness

6.3.1. Analysis of efficacy planned in the protocol

VE will be evaluated at the end of study period. VE will be estimated as 1-RR where RR is the risk ratio (proportion of subjects reporting the disease in the vaccinated group over the proportion in the placebo group) together with 90% CIs.

Subjects with missing values of efficacy endpoints 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.

6.3.2. Additional considerations

All the efficacy objectives (i.e. primary and secondary) will be evaluated in the PPS.

For the primary efficacy endpoint, VE will be assessed as 1-RR, where RR is the ratio of proportion of subjects with shigellosis in the vaccinated group on the proportion of subjects with shigellosis in the placebo group. As per protocol definition, **vaccine efficacy in reduction of shigellosis** will be demonstrated if the lower limit of the 90% CI of VE estimated with Miettinen-Nurminen method is above zero (see Section 12.1 for methods of calculation of the 90% CI for VE).

This information will be complemented with Barnard test [Barnard, 1945, 1947, 1949] (BARNARD option of the EXACT statement in SAS FREQ procedure): the lower limit of the 90% Confidence Interval for VE calculated with Miettinen-Nurminen method will be above zero if the p-value of the one-sided Barnard test is below 5%.

A subset of specific efficacy objectives will be analyzed also using 85% CI, see more details in *Second Line Analysis* below.

The following calculation will be performed for all secondary efficacy endpoints, according to the nature of the data:

- In case of endpoints measured as proportion (endpoints from a. to g., k., and l. sub-endpoints in Section 4.3), VE and 90% CI will be calculated as described above in the primary endpoint.
- For count endpoint (i.e. total number of grade 3-5 stools) the RR will be estimated as the rate of grade 3-5 stools in vaccinated subjects divided by the rate of grade 3-5 stools in placebo. 90% CI for this rate ratio will be calculated from an unadjusted negative binomial model, to take into account possible over-dispersion of data (DIST=NB in GENMOD SAS procedure); unadjusted P-value from the associated two-tailed Wald will be reported.
- Continuous endpoints (i.e. weight of grade 3-5 stools per subjects, weight of grade 3-5 stools per subject in those with at least a grade 3-5 stools, and weight of grade 3-5 stools accumulated from challenge to discharge per subject) will be compared using unequal variance two-sample t-test in case of normality of data, unequal variance

two-sample t-test on the log values in case log-values are normally distributed, or by Wilcoxon 2-sample test otherwise. In case of comparison via log-values, the geometric mean will be calculated as well as the arithmetic mean and associated 90% CI. Unadjusted P-values from two-tailed test will be considered reported.

- Time to event endpoint (i.e. days from challenge administration to onset of shigellosis) will be visually depicted by a Kaplan-Meier cumulative incidence curves, and compared using the Log-rank test. Unadjusted P-value will be reported. Day of challenge administration will be considered as Day 1.

Sensitivity analysis

The analysis of the primary objective will be repeated using FAS, instead of PPS.

The analysis for the following objectives will be repeated using FAS, instead of PPS, only in case the difference between FAS and PPS is greater than 10%:

- secondary efficacy objective of Shigellosis, as defined by CHIM expert working groups
- secondary efficacy objective of Shigellosis, as defined in Section 4.3
- secondary efficacy objective of More Severe Shigellosis, as defined in Section 4.3
- secondary efficacy objective of More Severe Diarrhea

Second line Analysis

The analysis for the following objectives will be repeated using a confidence level of 85% in the PPS:

- primary efficacy objective (i.e. assessing reduction of shigellosis)
- secondary efficacy objective of Shigellosis, as defined by CHIM expert working groups
- secondary efficacy objective of Shigellosis, as defined in Section 4.3
- secondary efficacy objective of More Severe Shigellosis, as defined in Section 4.3
- secondary efficacy objective of More Severe Diarrhea

Subgroup analysis

The analysis for primary efficacy objective will be repeated for the two subgroups of subjects who were responders or non-responders at the pre-challenge visit immunogenicity assessment.

6.3.3. Definition of shigellosis according to CHIM Expert Working Group

After protocol approval, a specific definition of shigellosis proposed by the CHIM Expert Working Group consensus meeting, different from the one defined as primary efficacy objective, was explicitly requested by the Bill and Melissa Gates Foundation (BMGF) and therefore added among the secondary efficacy objectives/endpoints.

BMGF shigellosis definition is the following:

- Moderate diarrhea OR severe diarrhea OR dysentery

With moderate diarrhea, severe diarrhea, and dysentery specifically defined as per Table 6.

Table 6 CHIM Expert Working Group Diarrhea and Dysentery definitions

Primary Endpoint	Definition
Severe Diarrhea	≥6 loose stools* in 24 hours OR >800 grams loose stools* in 24 hours
Moderate Diarrhea	[4-5 loose stools* in 24 hours OR 400-800 grams loose stools* in 24 hours] AND [oral temperature ≥38.0˚C OR ≥1 moderate constitutional/enteric symptom‡ OR ≥2 episodes of vomiting in 24 hours]
Dysentery	≥2 loose stools* with gross blood (hemoccult positive) in 24 hours AND [oral temperature ≥38.0˚C OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours]

* grade 3 to 5 stools
‡Nausea, abdominal pain/cramping, myalgia/arthralgia, malaise

6.4. Immunogenicity

6.4.1. Analysis of immunogenicity planned in the protocol

Analysis of binary variables:

The number and percentages of subjects will be summarized. Two-sided 95% CIs for the percentages will be computed.

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.

Analysis of continuous variables:

Antibody concentration below the limit of quantification (i.e. 22 EU/ml for ELISA and IC50 of 100 for L-SBA) in the respective assay will be set to half the limit for the

purposes of analysis. The limit will be calculated as the average limit of quantification for the assays done on Day 1 sera. The antibody concentrations/titers will be logarithmically transformed (base10) to fulfil the normal distribution assumption. GMC/GMTs will be calculated, with their associated two-sided 95% CIs, by exponentiating the corresponding log-transformed means and their 95% CI.

Additionally, within-subject geometric mean ratios (GMRs) will be computed for geometric mean concentrations at each time point after vaccination versus baseline (Day 1) and after challenge versus pre-challenge (Day 57). The GMRs and 95% CIs will be constructed by exponentiating the mean within-subject differences in log-transformed titers and the corresponding 95% CIs. GMR will be calculated only for subjects with valid baseline or pre-challenge valid immunogenicity results.

6.4.2. Additional considerations

The PPS will be the primary analysis set for the immunogenicity objectives; however, immunogenicity objectives will be evaluated also in the FAS, only in case the difference between FAS and PPS is greater than 10%.

The percentage of subjects with seroresponse by titer at baseline and with post vaccination antibody level ≥ 121 IgG units and associated two-sided 95% Clopper-Pearson CIs will be computed by vaccine group at each visit.

Unadjusted GMC/GMT s and associated two-sided 95% CIs will be computed for each group (i.e. vaccine and placebo) at each visit (i.e. Visit 1-Day 1, Visit 2-Day 8, Visit 3-Day 29, Visit 4-Day 36, Visit 5-Day 57, End of inpatient stay-Day 64, and Visit 6-Day 85). For each group, unadjusted GMC/GMTs and their 95% CIs will be obtained by exponentiating (base 10) the means and the lower and upper limits of the 95% CIs of the log-transformed concentrations (base 10). Additionally, within-subject unadjusted GMRs and associated two-sided 95% CIs will be computed for Day 8, Day 29, Day 36, and Day 57, respectively versus baseline and for Day 64 and Day 85 respectively versus Day 57 (pre-challenge).

For further details on standard business rule for GMC/GMT/GMR computation, see Section 12 Annex 1 standard data derivation rule and statistical methods.

Reverse Cumulative Distribution Curves

The distribution of antibody concentrations will be displayed using Reverse Cumulative Distribution Curves (RCDC) by visit (Day 1, Day 8, Day 29, Day 38, Day 57, Day 64, and Day 85) for groups of subjects receiving vaccine and placebo [i.e. a plot for each of the 7 time-point reporting two curves, one for vaccine group and one for placebo group].

RCDC will be produced to display also the cumulative weight of grade 3-5 stools and the number of grade 3-5 stools accumulated from challenge to discharge.

Derivation of immunogenicity data

a. Screening (anti *S. sonnei* LPS IgG Screening-ELISA)
   - The cut-off value for the anti *S. sonnei* LPS IgG Screening-ELISA is defined by the local laboratory.
   - A seronegative subject is a subject whose titer is below the cut-off value.
   - A seropositive subject is a subject whose titer is greater than or equal to the cut-off value.

b. Immunogenicity (anti S. sonnei LPS IgG-ELISA):
   - The cut-off value for the anti S. sonnei LPS IgG-ELISA is defined by the GVGH laboratory.
   - Subjects will be grouped in the following groups:
     - **Subjects with antibodies at baseline below Lower Limit of Quantification** (i.e. with antibodies at baseline ≥ Lower Limit of quantification, LLOQ (i.e. 22 EU/ml for ELISA and IC50 of 100 for L-SBA))
     - **Subjects without antibodies at baseline below Lower Limit of Quantification** (i.e. with antibodies at baseline < LLOQ)

Please note the following difference: seronegative at baseline is evaluated at the screening visit by the center lab and it is a reason for exclusion; while antibodies above/below LLOQ at baseline are evaluated by GVGH lab, and will determine subgroups to be analyzed.

All the immunogenicity analyses (ELISA and L-SBA) will be stratified by the above subgroups (i.e. presented overall, for subjects above LLOQ at baseline, and for subjects below above LLOQ at baseline).

Seroresponse is aimed to define a significant increase in anti *S. sonnei* LPS IgG concentration in post-vaccination samples; it is defined in this study as follows:

- If the baseline value is greater than 50 EU/mL 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/mL then an increase of at least 25 EU/mL in the post-vaccination sample as compared to baseline [i.e. (Post-vac minus baseline) ≥ 25 EU].

Note: Threshold values/increases given in the definition of seroresponse might be subject to change during the course of the study (e.g., in case of optimization/qualification/validation of the anti *S. sonnei* LPS-IgG-ELISA).

Additionally, all the analysis above described for immunogenicity by ELISA, will be repeated by subgroups defined by subjects who did or did not develop shigellosis (primary endpoint), and Shigellosis as defined by CHIM (secondary endpoint a.).

6.5. Analysis of safety

6.5.1. Analysis of safety planned in the protocol

The primary analysis will be performed on the All Exposed Set.

Analysis of solicited local and systemic adverse events and other reactions

Solicited AEs will be summarized according to defined severity grading scales.

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

Post-vaccination/challenge solicited AE reported from Day 1 to 7 days post-vaccination/challenge will be summarized by maximal severity and by vaccine/placebo group. The severity of solicited local AE erythema and induration will be summarized according to categories based on linear measurement.

Injection site pain and systemic reactions occurring up to 7 days after each vaccination will be summarized according to “mild”, “moderate” or “severe”.

Each solicited local and systemic AE will also be further summarized as “none” versus “any”; same summary will be reported for all local together, all systemic together, and local and systemic.

Analysis of unsolicited AEs

All the AEs occurring during the study, judged either as probably related, possibly related, or not related to vaccination/challenge by the investigator, will be recorded.

The original verbatim terms used by investigators to identify AEs in the eCRFs will be mapped to preferred terms using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary. The AEs will then be grouped by MedDRA preferred terms into frequency tables according to system organ class and PT. All reported AEs, as well as AEs judged by the investigator as at least possibly related to study vaccine/placebo or challenge agent, will be summarized according to system organ class and preferred term within system organ class. These summaries will be presented by vaccination group. When an AE occurs more than once for a subject, the maximal severity and strongest relationship to the vaccine/placebo group will be counted.

Separate summaries will be produced for the following categories:

a. SAEs
b. AESIs
c. AEs that are possibly or probably related to vaccine/placebo or challenge agent.
d. AEs leading to withdrawal from the study.

e. All AEs occurring within 28 days following administration of vaccination or challenge

Data listings of all AEs will be provided by subject.

In addition, AEs in the categories above will be provided as listed data.

6.5.2. Additional considerations

Standard data derivation procedures for safety is available in Section 12.2.5.

6.5.2.1. Exclusion of implausible solicited Adverse Event

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 7.

Table 7 Implausible Solicited Adverse Events

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

6.5.2.2. Solicited Adverse Events

All analyses will be based on the 'as treated' Solicited Safety Set (see Section 12.2.5).

Post-vaccination Solicited Adverse Events will be reported from 30 minutes until 7 days after vaccination using structured diary cards. The analyses of solicited adverse events will be done separately for 30 minutes, for the remaining day, and after that, for each day between day 2 until day 7 included. In addition solicited adverse events ongoing after day 7 will be presented as unsolicited AE.

The list of Solicited Local and Systemic adverse events is reported in the Table 8.

Table 8 List of Local and Systemic solicited adverse events

Local adverse events
Pain at injection site Erythema at injection site Induration at injection site
Systemic adverse events
Arthralgia Chills Fatigue Malaise Myalgia Fever Headache

The maximum intensity of local injection site erythema/induration/fever will be scored at GSK Biologicals as in Table 9 and Table 10.

Table 9 Intensity grade for Erythema/Induration

	Erythema/Induration
0:	< 25 mm
1:	≥ 25 - ≤ 50 mm
2:	> 50 -≤ 100 mm
3:	> 100 mm

Table 10 Intensity grade for fever

Fever			
Grade 0 Absent	**Grade 1 Mild**	**Grade 2 Moderate**	**Grade 3 Severe**
< 38.0°C	≥ 38.0°C – < 39.0°C	≥ 39.0°C – <40.0°C	≥ 40.0°C

A list of Solicited AE be assessed after vaccination or after challenge agent administration with associated intensity is reported in the Table 11. Note that the AEs marked with # will be evaluated after both vaccination and challenge.

Table 11 Intensity scales for solicited symptoms

Adverse Event	Intensity grade	Parameter
After vaccine/placebo administration		
Pain at injection site	0	Absent
	1	Easily tolerated.
	2	Interferes with normal activity
	3	Prevents normal activity.
Erythema at injection site		Record greatest surface diameter in mm
Induration at injection site		Record greatest surface diameter in mm
Fever#*		Record temperature in °C/°F
Headache#	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
Fatigue#	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
Arthralgia#	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
Malaise#	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
Myalgia#	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
Chills	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
After challenge agent administration		
Nausea	0	Absent
	1	Mild or transient; maintains reasonable intake
	2	Moderate discomfort; intake decreased significantly; some activity limited
	3	No significant intake and requires medical intervention
	4	Hospitalization required
Abdominal cramping	0	Absent
	1	No interference with daily activities
	2	Some interference with daily activities not requiring medical intervention
	3	Prevents daily activities and requires medical intervention
	4	ER visit or hospitalization
Abdominal pain	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
Gas	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity

Adverse Event	Intensity grade	Parameter
Anorexia	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
Vomiting	0	Absent
	1	One episode within a 24-hour period
	2	2 episodes within a 24-hour period
	3	>2 episodes within a 24-hour period or requires medical intervention
	4	ER visit or hospitalization for hypotensive shock
Diarrhea**	0	Absent
	1	2-3 Grade 3-5 stools (loose or watery) or <400 g/Grade 3-5 (loose or watery) stools per 24 hours
	2	4-5 Grade 3-5 stools (loose or watery) or 400-800 g/ (loose or watery) Grade 3-5 stools per 24 hours
	3	6 or more Grade 3-5 stools (loose or watery) or >800 g/Grade 3-5 (loose or watery) stools per 24 hours or requires medical intervention
	4	≥ 10 loose stools (Grade 3 to 5) or ≥ 1000 grams of Grade 3 to 5 stools within 24 hours

#Same grading is used for events present both post-vaccination and post-challenge.
*Fever is defined as temperature ≥ 38.0°C / 100.4°F. The preferred location for measuring temperature in this study will be the oral cavity.
**The end of a diarrheal episode occurs when a volunteer does not pass any Grade 3-5 stool within 24 hours.
ER: emergency room.

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

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 from 30 Min, and specifically for each day between day 2 through Day 7.
4. Solicited adverse events and indicators of solicited adverse events, occurrence of at least one event by category (local, systemic) and interval from 30 Min through 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 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.

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: Number of days with solicited adverse events

The number of days with the adverse event is defined irrespective of severity. This means at least 'mild' solicited adverse event that are assessed qualitatively and ≥ 25 mm for erythema and induration. If a solicited adverse event continues beyond day 7 the period after day 7 is added.

Level 5: 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" (≥ 25 mm for erythema and induration) for a subject if he/she reports greater than "none" 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 from 30 min to day 7.

6.5.2.3. Unsolicited Adverse Events

All the unsolicited adverse events occurring within 28 days following administration of each vaccination or occurring within 28 days following challenge administration, judged either as probably related, possibly related, or not related to vaccination by the investigator, will be recorded. 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 and PT. 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 9.1.4 of protocol for definition) will be analyzed, i.e., excluding those after a subject has given informed consent but before vaccination: those events will be listed with an appropriate flag.

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

- Onset between first vaccination and second vaccination.
- Onset between second vaccination and challenge administration.

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

During the course of this study, symptomatic neutropenia will be considered as AESIs.

Table 12 List of potential AESI to be followed during the study

Blood disorders
• Symptomatic neutropenia (all Grades)

Neutropenia is defined as decrease of neutrophil count asymptomatically or symptomatically. Available local laboratory ranges will be used to define neutropenia. This is completely diagnosed by laboratory testing for complete blood count. However only symptomatic neutropenia cases will be considered as AESI and reported as such.

The following grading will be used to classify neutropenia:

- Grade 1: 1800-1500 cells/µL.
- Grade 2: 1499-1000 cells/µL.
- Grade 3: 999-500 cells/µL.
- Grade 4: < 500 cells/µL.

In performing their assessment of symptomatic neutropenia cases, investigators are strongly advised to use Table 13 below.

Table 13 Evaluation of symptomatic neutropenia

Common presenting symptoms of neutropenia	Physical findings on examination of a subject with neutropenia
Low-grade fever	Fever
Sore mouth	Stomatitis
Odynophagia	Periodontal infection
Gingival pain and swelling	Cervical lymphadenopathy
Skin abscesses	Skin infection: The skin examination focuses on rashes, ulcers, or abscesses
Recurrent sinusitis and otitis	Splenomegaly
Symptoms of pneumonia (e.g., cough, dyspnea)	Associated petechial bleeding
Perirectal pain and irritation	Perirectal infection
Neutropenic sepsis	**Other according to Investigator's opinion**
Neutropenic infection	
Neutropenic colitis	
Other according to Investigator's opinion	

In order to facilitate the documentation of AESI in the eCRF, a list of MedDRA preferred terms (PTs) and PT codes corresponding to the above diagnoses will be available to investigators at study start.

When there is enough evidence to make any of the above diagnoses, the AE must be reported as AESI. Symptoms, signs or conditions which might (or might not) represent the above diagnoses, should be recorded and reported as AEs but not as AESI until the final or definitive diagnosis has been determined, and alternative diagnoses have been eliminated or shown to be less likely.

6.5.2.4. 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 as per the following codes:

Solicited symptom	Local/Systemic symptom	Lower level term code	Corresponding Lower level term decode
Erythema at injection site	local	10015150	Erythema
Induration at injection site	local	10060708	Induration
Pain at injection site	local	10022086	Injection site pain
Arthralgia	systemic	10003239	Arthralgia
Chills	systemic	10008531	Chills
Fatigue	systemic	10016256	Fatigue
Fever	systemic	10016558	Fever
Headache	systemic	10019211	Headache
Malaise	systemic	10025482	Malaise
Myalgia	systemic	10028411	Myalgia

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 preferred terms and according to occurrence of each event.

6.5.2.5. Clinical Safety Laboratory Investigations

The investigator must assess all safety laboratory results (see **Section 9.1.4** of the study protocol). Clinically significant modifications in hematology test values will be assessed by medical judgment based on interpretation of deviations from the institution's normal values. Abnormal laboratory findings (e.g., clinical chemistry, hematology, urinalysis) or other abnormal assessments that are judged by the investigator to be clinically significant will be recorded as AE or SAE if they meet the definition of an AE or SAE (refer to **Sections 9.1.1** and **9.1.2** of the Study Protocol).

Clinically significant abnormal laboratory findings or other abnormal assessments that are present at baseline and significantly worsen following the start of the study will also be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgement in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant. Clinically significant modifications in hematology will be assessed by medical judgment based on interpretation of deviations from institution's normal values and recommendations from CBER (FDA Center for Biologics Evaluation and Research) FDA GUIDANCE FOR INDUSTRY: Toxicity Grading Scale for Healthy Adults and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials and predefined list of sign and symptoms related to neutropenia.

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 (4 x 3 shift tables, including missing at baseline as a category).

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.

6.5.2.6. Concomitant Medication

Medications will be coded using the GSKDRUG dictionary.

The frequencies and percentages of subjects starting/reporting concomitant medications/products/vaccination during all study period will be tabulated by vaccine group for each study dose and across doses. See Section 7.6.1 of the Study Protocol for the list of medications/products/vaccination recorded in the eCRF.

An overview of the protocol-required reporting periods for AEs, SAEs, and pregnancies is given below.

Table 14 Reporting periods for collecting safety information

Timepoints	Scr	D1	D8	D29	D36	D57	D57-D64	D85	D237 (M8)
Solicited local and systemic AEs									
Unsolicited AEs									
AEs/SAEs leading to withdrawal from the study									
AESI SAEs									
Pregnancies									
SAEs related to study participation or concurrent GSK medication/vaccine									

Scr: Screening
D: Day
M: Month

7. ANALYSIS INTERPRETATION

Except for analyses on objectives with a pre-defined success criterion and an appropriate type I error control (see Section 3.1), comparative analyses will be descriptive with the aim to characterize the difference in reactogenicity and immunogenicity efficacy between groups. These descriptive analyses should not be interpreted.

8. CONDUCT OF ANALYSES

8.1. Sequence of analyses

Description	**Analysis ID**	**Disclosure Purpose (CTRS=public posting, SR=study report, internal)**	**Dry run review needed (Y/N)**	***Study Headline Summary (SHS)*requiring expedited communication to upper management (Yes/No)**	**Reference for TFL**
Interim Efficacy Analysis	E1_01	Efficacy results to BMGF for funding Shigella 4-C.	Yes	Yes	Section 2 of TFL TOC.
Interim Immunogenicity Analysis	E1_06	Immunogenicity results to BMGF	Yes	No	Section 3 of TFL TOC.
Final Analysis	E1_02	SR, CTSR	Yes	No	Section 4 of TFL TOC.

The analysis for the Independent Data Monitoring Committee (IDMC) is planned to be performed by an Independent Data Analysis Center (IDAC). This analysis is planned as per IDMC Charter and will be provided three times, once after each of the first three cohorts (36 subjects expected) have completed the challenge phase of the trial.

One Interim Efficacy Analysis is planned after the last subject of the last cohort had been discharged and will include all post-challenge efficacy objectives on all subjects. Tables with efficacy results will be produced by unblinded vaccine and placebo groups, but no listings will be provided. Interim Efficacy Analysis will be integrated at the end of the study with unblinded safety listings and with Efficacy Analysis stratified by antibody concentration below the limit of quantification at baseline visit and by seroresponse at pre-challenge.

One Interim Immunogenicity Analysis is planned after latest immunogenicity lab results (ELISA and L-SBA) had been released. This analysis will include all secondary immunogenicity data (i.e. ELISA) plus SBA tertiary immunogenicity data of all subjects (all 4 cohorts). Tables with immunogenicity results will be produced by unblinded vaccine and placebo groups, but no listings will be provided. Interim ELISA immunogenicity analyses will be also repeated by subgroups defined by subject who did or did not develop shigellosis (primary endpoint), and Shigellosis as defined by CHIM (secondary endpoint a.).

An integrated clinical study report containing all Statistical Analysis Results will be written and made available to the investigators after the final analysis.

If the data for tertiary endpoints become available at a later stage, (an) additional analysis/analyses will be performed. These data will be documented in annex(es) to the study report and will be made available to the investigators at that time.

8.2. Statistical considerations for interim analyses

No statistical adjustment will be made for the interim analyses. The need of the interim analysis on efficacy is that it serves as Go/No Go decision for start of the 4-component Shigella vaccine trial.

9. CHANGES FROM PLANNED ANALYSES

To complement the immunogenicity profile, the following timepoints were added to the immunogenicity analysis:

- Seroresponse and concentration ≥ 121 EU at 7 days after each vaccination.
- Concentration ≥ 121 EU at 7 and 28 days after challenge.

To complement the immunogenicity profile, the immunogenicity analyses were repeated in the following subgroups:

- In subject who did and did not developed shigellosis (primary endpoint)
- In subject who did and did not developed shigellosis as defined by CHIM (secondary endpoint a.)

10. NON-STANDARD DATA DERIVATION RULES AND STATISTICAL METHODS

The following sections describe additional derivation rules and statistical methods which are not presented in section 12.1.

10.1. Data derivation

Specific data derivation will be put in place to derive the primary and the secondary efficacy endpoints before Database freeze. Details are reported in Section 12.2.7.

10.2. Statistical Method

The Confidence Interval for Vaccine Efficacy estimated with Miettinen-Nurminen method for the primary objective will be complemented with Barnard test [Barnard,1945, Barnard,1947, Barnard,1949] (BARNARD option of the EXACT statement in SAS FREQ procedure).

The lower limit of the 90% Confidence Interval for VE calculated with Miettinen-Nurminen method will be above zero if the p-value of the one-sided Barnard test is below 5%.

11. LIST OF FINAL REPORT TABLES, LISTINGS AND FIGURES

The TFL TOC including the mock-up tables can be found in eTMF folder Section 11.1.1.

12. ANNEX 1 STANDARD DATA DERIVATION RULE AND STATISTICAL METHODS

12.1. Statistical Method References

The 90% CIs for RRs calculated in the efficacy analyses (and the correspondent 90% CI for VE, defined as 1-RR) will be calculated using the Miettinen-Nurminen method [Miettinen, 1985].

The exact two-sided 95% CIs for a proportion within a group will be the Clopper-Pearson exact CI [Clopper, 1934].

12.2. Standard data derivation

The v15 GSK standard data derivation will be adopted. However, custom programming will be implemented if needed.

12.2.1. Date derivation

- SAS date of birth derived from a character date: In case day is missing, 15 is used. In case day & month are missing, 30June is used. This is also applicable to start date of Ae and medication.
- Onset day for an event (ae, medication, vaccination, …): The onset day is the number of days between the last study vaccination & the onset/start date of the event. This is 1 for an event starting on the same day as a vaccination. See SAS date derived in case the start date of the event is incomplete.
- Duration: Duration of an event is expressed in days. It is the number of days between the start & the stop dates + 1. Therefore duration is 1 day for an event starting & ending on the same day.
- Association of an event to the specific epoch: An adverse event belongs to Epoch 002, if the onset date is before and excluding Visit 5 or the last contact date (assuming last contact date is before Visit 5), whichever is coming first. An adverse event belongs to Epoch 003, if the onset date is subsequent to and including Visit 5 or the last contact date (assuming last contact date is at Visit 5 or at a subsequent date), whichever is coming first.

12.2.2. Dose number

- The study dose number is defined in reference to the number of study visits at which vaccination occurred. More specifically dose 1 refers to all vaccines administered at the first vaccination visit while dose 2 corresponds to all vaccinations administered at the second vaccination visit even if this is the first time a product is administered to the subject.
- Relative dose: the relative dose for an event (AE, medication, vaccination) is the most recent study dose given before an event. In case the event takes place on the day a study dose is given, the related dose will be that of the study dose, even if the event actually took place before vaccination. Otherwise, if the event actually took place the same day of vaccination and before vaccination, the related dose will be that of the previously administered dose.
- The number of doses for a product is the number of times the product was administered to a subject.
- The incidence per dose is the number of vaccination visits at which an event was reported among all vaccination visits.

12.2.3. Demography

- Age: Age at the reference activity, computed as the number of days between the date of birth and the reference activity and converted in months (= days *12 /365.25) (keeping 1 decimal digit).
- Conversion of weight to kg

  The following conversion rule is used:

  – Weight in Kilogram= weight in Pounds / 2.2

  – Weight in Kilogram =weight in onces / 35.2

  The result is rounded to 2 decimals.

- Conversion of height to cm

  The following conversion rule is used:

  – Height in Centimetres = Height in Feet * 30.48

  – Height in Centimetres = Height in Inch * 2.54

  The result is rounded to the unit (ie no decimal).

- Conversion of temperature to °C

  The following conversion rule is used:

  – Temperature in °Celsius = ((Temperature in °Fahrenheit -32) *5)/9

  The result is rounded to 1 decimal.

12.2.4. Immunogenicity

- For a given subject and given immunogenicity measurement, missing or non-evaluable measurements will not be replaced. Therefore, an analysis will exclude subjects with missing or non-evaluable measurements.
- The Geometric Mean Concentrations (GMC) calculations are performed by taking the anti-log of the mean of the log titre transformations. Antibody titres below the cut-off of the assay will be given an arbitrary value of half the cut-off of the assay for the purpose of GMC calculation. Refer to Section 6.4.2 for details regarding cut-off definition and calculations.
- All CI computed will be two-sided 95% CI.

12.2.5. Safety

- For the analysis of solicited symptoms, missing or non-evaluable measurements will not be replaced. Therefore, the analysis of the solicited symptoms based on the Exposed Set will include only vaccinated subjects for doses with documented safety data (i.e., symptom screen completed). This corresponds to the Solicited Safety Set defined in the protocol (Section 11.5.3.1). More specifically the following rules will be used:
  - Subjects who documented the absence of a solicited symptom after each of vaccine dose will be considered not having that symptom after that dose.
  - Subjects who documented the presence of a solicited symptom and fully or partially recorded daily measurement over the solicited period will be included in the summaries at that dose and classified according to their maximum observed daily recording over the solicited period.
  - Subjects who documented the presence of a solicited symptom after one dose without having recorded any daily measurement will be assigned to the lowest intensity category at that dose (i.e., 38.0°C for fever or grade 1 for other symptoms).
  - Doses without symptom sheets documented will be excluded.
- For analysis of unsolicited adverse events, such as serious adverse events or adverse events by primary MedDRA term, and for the analysis of concomitant medications, all vaccinated subjects will be considered. Subjects who did not report the event or the concomitant medication will be considered as subjects without the event or the concomitant medication respectively. This corresponds to the Solicited Safety Set defined in the protocol (Section 11.5.3.2).

Note that for all tables described in this section, the way the percentage of subjects will be derived will depend on the event analyzed (see table below for details). As a result, the N value will differ from one table to another.

Event	N used for deriving % per subject for Vaccination phase	N used for deriving % per dose for Vaccination phase
Concomitant vaccination	All subjects with study vaccine administered	All study visits with study vaccine administered
Solicited general symptom	All subjects with at least one solicited general symptom documented as either present or absent (i.e., symptom screen completed)	All study visits with study vaccine administered and with at least one solicited general symptom documented as either present or absent (i.e., symptom screen completed)
Solicited local symptom	All subjects with at least one solicited local symptom documented as either present or absent (i.e., symptom screen completed)	All study visits with study vaccine administered and with at least one solicited local symptom documented as either present or absent (i.e., symptom screen completed)
Unsolicited symptom	All subjects with study vaccine administered	All study visits with study vaccine administered
Concomitant medication	All subjects with study vaccine administered	All study visits with study vaccine administered

12.2.6. Number of decimals displayed:

The following decimal description from the decision rules will be used for the demography, efficacy, immunogenicity and safety/reactogenicity.

Display Table	Parameters	Number of decimal digits
Demographic characteristics	Mean, median age	1
Demographic characteristics	SD (age)	1
Immunogenicity	Ratio of GMT/C	2
Reactogenicity	Mean, Min, Q1, Median, Q3, Max for duration	1
All summaries	% of count, including LL & UL of CI	1
All summaries	% of difference, including LL & UL of CI	2
All summaries	p-value	3

12.2.7. Efficacy

Specific data derivation will be put in place to derive the primary and the secondary efficacy endpoints before Database freeze.

An overview of the algorithms to be used to derive all efficacy endpoints is reported in Table 15.

Table 15 Derivation of efficacy endpoints

Endpoint	Definition / Derivation
Primary Efficacy	
Shigellosis	[Shedding of S. sonnei strain 53G] AND
	[(Moderate diarrhea) OR
	(Severe diarrhea) OR
	(oral temperature ≥ 38.5°C)]
Derivation of Shigellosis	
Moderate diarrhea	[4 to 5 stools[1] within 24 hours] OR

Endpoint	Definition / Derivation
	[400 to 800 grams of stools[1] within 24 hours]
Severe diarrhea	[≥ 6 stools[1] within 24 hours] OR
	[> 800 grams of stools[1] within 24 hours] OR
	[intravenous fluids administration, i.e. ATC B05XA in concomitant medication] OR
	[anticipation of antibiotic treatment before the 5th day after challenge]
Secondary Efficacy	
a. Shigellosis as per CHIM definition	[Moderate diarrhea] OR
	[Severe diarrhea] OR
	[Dysentery]
Derivation of Shigellosis as per CHIM definition	
Moderate diarrhea	[(4 to 5 stools[1] within 24 hours) OR
	(400 to 800 grams of stools[1] within 24 hours)]
	AND[2]
	[(oral temperature ≥ 38.0˚C) OR
	(≥1 constitutional/enteric symptom[3] graded ≥2) OR
	[≥2 episodes of vomiting within 24 hours])
Severe diarrhea	[≥6 stools[1] within 24 hours] OR
	[800 grams loose stools[1] within 24 hours]
Dysentery	[≥2 stools[1] with gross blood (hemoccult positive) in 24 hours]
	AND[2]
	[oral temperature ≥38.0˚C OR
	(≥1 constitutional/enteric symptom[3] graded ≥2) OR
	(≥2 episodes of vomiting in 24 hours)]
b. Shigellosis as defined by any one of the three following possible endpoints:	
	[Severe diarrhea] OR
	[Moderate diarrhea AND (oral temperature ≥38.0°C OR ≥1 at least moderate constitutional/enteric symptom[4])] OR
	[Dysentery]
Derivation of Severe diarrhea	
	[≥ 6 stools[1] within 24 hours] OR
	[> 800 grams of stools[1] within 24 hours]
Derivation of Moderate diarrhea AND (oral temperature ≥38.0°C OR ≥1 at least moderate constitutional/enteric symptom[4])	
	(
	[4 to 5 stools[1] within 24 hours] OR
	[400 to 800 grams of stools[1] within 24 hours]
	)
	AND[2]
	(
	(oral temperature ≥ 38.0˚C) OR
	>=1 at least moderate (i.e. >=grade 2) constitutional/enteric symptom[4])

Endpoint	Definition / Derivation
	)
Derivation of Dysentery	
	[≥2 stools[1] with gross blood (hemoccult positive) in 24 hours]
	AND[2]
	(>=1 at least mild (i.e. >=grade 1) constitutional/enteric symptom[4])
c. More Severe Shigellosis as defined by any one of the two following possible endpoints:	
	[(Moderate OR Severe diarrhea) AND (oral temperature ≥38.0°C OR ≥1 severe constitutional/enteric symptom)] OR
	[Dysentery AND (oral temperature ≥38.0°C OR ≥1 severe constitutional/enteric symptom[4])]
Derivation of Severe diarrhea	
	[≥ 6 stools[1] within 24 hours] OR
	[> 800 grams of stools[1] within 24 hours]
Derivation of Moderate diarrhea	
	[4 to 5 stools[1] within 24 hours] OR
	[400 to 800 grams of stools[1] within 24 hours]
Derivation of oral temperature ≥38.0°C	
	(oral temperature ≥ 38.0˚C)
Derivation of ≥1 severe constitutional/enteric symptom)	
	>=1 at least severe (i.e. >=grade 3) constitutional/enteric symptom[4])
Derivation of Dysentery	
	[≥2 stools[1] with gross blood (hemoccult positive) in 24 hours]
d. Shedding of *S. sonnei* strain 53G	At least one stool[1] sample positive to *S. sonnei* strain 53G either by culture or qPCR
e. Severe diarrhea	Severe diarrhea as defined under primary endpoint
f. More severe diarrhea	[≥ 10 stools[1] within 24 hours] OR
	[≥ 1000 grams of stools[1] within 24 hours]
g. Dysentery	[≥2 stools[1] with gross blood (hemoccult positive) in 24 hours]
	AND[2]
	(≥1 constitutional/enteric symptom[5] at least mild (i.e. >=grade 1))
h. weight of grade 3-5 stools	weight of each stool[1] during follow-up
i. weight of grade 3-5 stools accumulated from challenge to discharge	For each subject sum the weight of each stool[1] accumulated from challenge to discharge
j. number of grade 3-5 stools	For each subject count the number of stools[1] accumulated from challenge to discharge
k.	[At least one stool[1] sample positive to *S. sonnei* strain 53G either by culture or qPCR]
	AND[2]
	[(Moderate diarrhea) OR
	(Severe diarrhea) OR
	(Dysentery as defined in g.) OR
	(oral temperature ≥ 38.5°C) OR
	(≥1 intestinal symptom[6] graded ≥3)]

Endpoint	Definition / Derivation
l.	
1	[At least one stool (whichever is the grading)]
	AND[2] NOT
	[(Moderate diarrhea) OR
	(Severe diarrhea) OR
2	≥1 abdominal pain graded ≥1
3	≥1 abdominal cramps graded ≥1
4	≥1 abdominal gas graded ≥1
5	≥1 abdominal anorexia graded ≥1
6	≥1 abdominal nausea graded ≥1
7	≥1 abdominal headache graded ≥1
8	≥1 abdominal myalgia graded ≥1
9	≥1 abdominal malaise graded ≥1
10	≥1 abdominal arthralgia graded ≥1
11	≥1 oral temperature ≥ 38.5°C
12	≥1 vomit graded ≥1
13	≥1 IV fluid administration
m. Time to onset of Shigellosis during the post-challenge period	Shigellosis is defined as in primary objective. The day associated to the first shigellosis[6] is the time of onset of Shigellosis.

[1]Considering only stools graded 3-5
[2]Where AND combines conditions occurring in the 8-day post-challenge inpatient period
[3]Where constitutional/enteric symptoms [symptoms list 1] are: nausea, abdominal pain, abdominal cramping, myalgia, arthralgia, malaise
[4]Where constitutional/enteric symptoms [symptoms list 2] are: headache, fatigue, arthralgia, malaise, myalgia, chills, nausea, abdominal cramping, abdominal pain, gas, anorexia, vomiting
[5]Where constitutional/enteric symptoms [symptoms list 3] are: headache, fatigue, arthralgia, malaise, myalgia, chills, nausea, abdominal cramping, abdominal pain, gas, anorexia, vomiting, and fever
[6]Where intestinal symptoms [symptoms list 4] are: abdominal pain, abdominal cramping, gas, nausea, vomiting, anorexia
If the conditions defining shigellosis are verified in 2 different days (e.g. shedding at day 1 and Severe Diarrhea at day 3), the day of onset of Shigellosis is defined as the day when the latest condition is met is (e.g. day 3).
If a 24-hour period where Shigellosis definition is fulfilled covers 2 subsequent days (e.g. start day is day 2 and end day is day 3) the day associated to onset of shigellosis will be the one associated to start day.

13. ANNEX 2: SUMMARY ON ELIMINATION CODES

Please refer to the exclusion matrix stored in LSAF for a comprehensive list of exclusion codes among all the analysis sets.

14. ANNEX 3: STUDY SPECIFIC MOCK TFL

Please refer to TFL TOC word file in eTMF folder Section 11.1.1 for the study specific mocks.

The data display, title and footnote are for illustration purpose and will be adapted to the study specificity as indicated in the TFL TOC. Note that there may be few changes between the study specific SAP mock TFL and the final TFLs as editorial/minor changes do not require a SAP amendment

15. REFERENCES

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gsk GlaxoSmithKline	**Statistical Analysis Plan**
Detailed Title:	A phase IIb, randomized, placebo-controlled, single center, observer-blind, human-challenge study to evaluate the efficacy, safety and immunogenicity of 2 vaccinations with GVGH *Shigella sonnei* vaccine (1790GAHB) administered by intramuscular route in healthy non-immune adult population.
eTrack study number and Abbreviated Title	205626 (S SONNEI MONO GMMA SBVGH-003 [H03_03TP])
Scope:	All primary and secondary objectives pertaining to the above study. The Analysis Plan for the tertiary objectives is part of a separate SAP.
Date of Statistical Analysis Plan	Final: 09-Aug-2018
Co-ordinating author:	PPD (GVGH Expert Biostatistician)
Reviewed by:	PPD (GVGH Head of Clinical Development and Regulatory Affairs) PPD (GVGH Project Physician) PPD (Lead statistician) PPD (Lead statistical Analyst) PPD (Public disclosure representative)
Approved by:	PPD (GVGH Head of Clinical Development and Regulatory Affairs) PPD (Lead statistician) PPD (Lead statistical Analyst) PPD (Lead Scientific Writer)

APP 9000058193 Statistical Analysis Plan Template (Effective date: 14 April 2017)

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LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Events of Special Interest
BMGF	Bill and Melissa Gates Foundation
CBER	FDA Center for Biologics Evaluation and Research
CHIM	Controlled Human Infection Model
CI	Confidence Interval
CTRS	Clinical Trial Registry Summary
eCRF	electronic Case Report Form
ELISA	Enzyme-linked immunosorbent assay
EoS:	End of Study
ER	Emergency Room
ES	Exposed Set
EU/ml	ELISA unit per milliliter
FAS	Full Analysis Set
Fc	Fragment crystallisable
FDA	Food and Drug Administration, United States of America
GMC	Geometric mean antibody concentration
GMMA	Generalized Modules for Membrane Antigens
GMR	Geometric Mean Ratio
GSK	GlaxoSmithKline
GVGH	GSK Vaccines Institute for Global Health
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HLA	Human Leukocyte Antigen
ICF	Informed Consent Form
IDAC	Independent Data Analysis Center
IDMC	Independent Data Monitoring Committee
IgG	Immunoglobulin G
IM	Intramuscular
LL	Lower Limit of the confidence interval
LPS	Lipopolysaccharide

MedDRA	Medical Dictionary for Regulatory Activities
MSD	Moderate to Severe Diarrhea
OAg	O antigen
PBMC	Peripheral Blood Mononuclear Cells
PCD	Primary Completion Date
PD	Protocol Deviation
PPS	Per Protocol Set
qPCR	quantitative Polymerase Chain Reaction
RCDC	Reverse Cumulative Distribution Curve
RR	Risk Ratio
S. sonnei	Shigella sonnei
SAE	Serious adverse event
SAP	Statistical Analysis Plan
SBA	Serum Bactericidal Assay
SBIR	GSK Biological's Internet Randomization System
SD	Standard Deviation
sIgA	Secretory Immunoglobulin A
TFL	Tables Figures and Listings
TOC	Table of Content
UL	Upper Limit of the confidence interval
Vacc	Vaccination
VE	Vaccine Efficacy

1. DOCUMENT HISTORY

Date	Description	Protocol Version
09-AUG-2018	Final Version	22-JUN-2018 Amendment 2 Final

2. STUDY DESIGN

This is a randomized, placebo-controlled, single center, observer-blind, phase 2b human challenge trial. The study includes a screening visit (performed between study Days -45 to -1), 2 clinical visits with vaccination (performed at study Day 1 and Day 29), 2 clinical visits 7 days after each vaccination (performed at study Day 8 and Day 36), 1 clinical visit with challenge administration 28 days after second vaccination (performed at study Day 57), and 3 visits after challenge, 28 days (study Day 85), 56 days (study Day 113) and 6 months (study Day 237) after challenge.

This study will evaluate the efficacy, safety and immunogenicity of the 1790GAHB vaccine administered via intramuscular (IM) route to adults (18-50 years of age at enrolment). The objectives will be assessed through the conduct of the study in two phases: a vaccination phase with 1790GAHB vaccine or placebo followed by a challenge phase with *S. sonnei* strain 53G.

A total of 72 subjects will be divided in 4 cohorts of 18 subjects each. The vaccination and challenge will be done in overlapping cohorts and randomization will ensure there is the same number of vaccinees and controls in each cohort. However if for any reason, a cohort did not reach the planned 18 subjects, additional subjects can be added to the following cohorts. The maximum number of subjects must not be more than 20 (maximum number of beds) in each cohort.

- **Experimental design:** Phase IIB, observer-blind, randomized, placebo control, mono-centric study with two parallel groups (*S. sonnei* group and placebo group).
- **Duration of the study**: Each subject will be followed up for approximately 6 months after challenge with the pathogenic *S. sonnei* strain 53G, with a total study duration of approximately 32 weeks (i.e., 8 months) for each study subject.
  - Epoch 001: Starting at Screening Visit (Day -45 to Day -1) and ending before Visit 1 (Day 1).
  - Epoch 002: Starting the day of randomization and first vaccination (Visit 1) and ending before the receipt of the challenge agent, 28 days after second vaccination (Visit 5).
  - Epoch 003: Starting with the receipt of the challenge agent at 28 days after second vaccination (Visit 5) and ending 6 months after challenge (Visit 8).
- **Primary completion date (PCD):** Visit 8 (6 months after challenge).

  Refer to Glossary Section of the protocol for the definition of PCD.

- **End of Study (EoS):** Last testing results released of samples collected for primary and secondary objectives at Visit 8.

  Refer to Glossary Section of the protocol for the definition of EoS.

- **Study groups:**

Table 1 Study groups and epochs foreseen in the study

Study groups	Number of subjects	Age (Min - Max)	Epochs		
			Epoch 001	Epoch 002	Epoch 003
			Screening	1st and 2nd vaccination	Challenge
S. sonnei	36	18 years – 50 years	x	x	x
Placebo	36	18 years – 50 years	x	x	x

Table 2 Study groups and treatment foreseen in the study

Treatment name	Vaccine/ placebo/ challenge agent name	Study Groups	
		S. sonnei	Placebo
S. sonnei	1790GAHB	x	
Placebo	GAHB-Placebo		x
Challenge	*S. sonnei* 53G strain	x	x

- **Control:** Placebo control with GAHB-Placebo with the same composition as the vaccine except the active ingredient. GAHB-Placebo will also be used as diluent for bed-side mixing of the vaccine.
- **Vaccination schedule:** Subjects will receive 2 doses of either the study vaccine or placebo 28 days apart. At 28 days after the second dose, all subjects will receive the challenge dose.
- **Treatment allocation:** Following the screening period before the first vaccination (Day -45 to Day -1), subjects will be randomized in a 1:1 ratio to receive either the study vaccine or the placebo.
- **Blinding:** The study will be observer-blind.

Table 3 Blinding of study epochs

Study Epochs	Blinding
Screening (Epoch 001)	N/A
1st and 2nd vaccination (Epoch 002)	observer-blind
Challenge (Epoch 003)	observer-blind

An overview of the study design is given in Figure 1 and a list of study procedures is reported in Table 4. For further details please refer to Section 3 of the protocol.

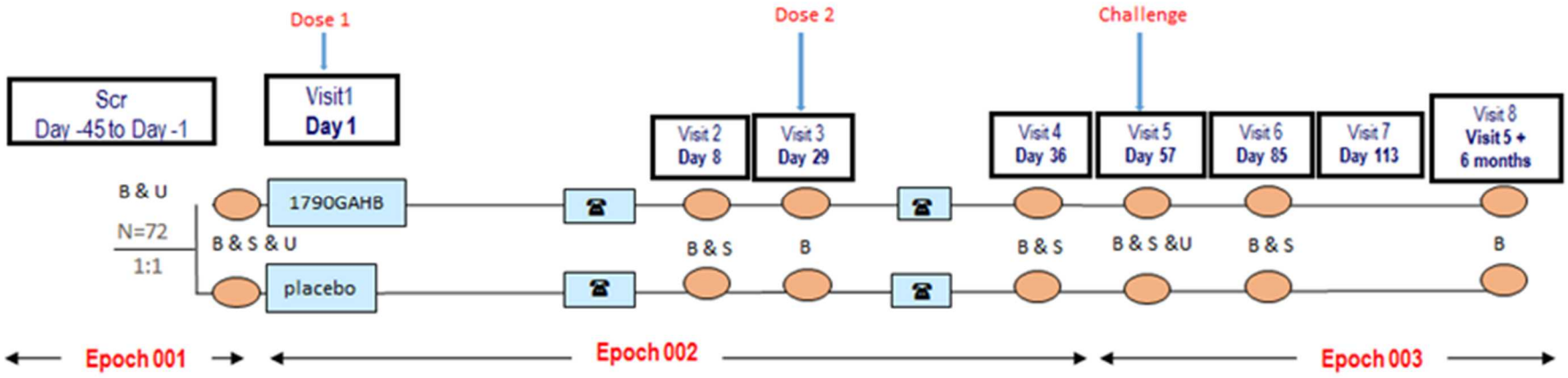
Randomization

After obtaining the signed and dated ICF from the subject and having checked the eligibility of the subject, the site staff in charge of the vaccine administration will access GSK Biological's Internet Randomization System (SBIR). Upon providing the subject identification number, the randomization system will determine the study group and will provide the treatment number to be used for each dose.

The number of each administered treatment must be recorded in the eCRF (electronic Case Report Form) on the Vaccine Administration screen.

The target will be to enroll approximately 72 eligible subjects (36 per treatment group) who will be randomly assigned to two study groups in a (1: 1) ratio. The randomization algorithm will use a minimization procedure accounting for study. The block size used for the material randomization list is 6.

Figure 1 Study Design



Scr: Screening
N: Number of subjects
B: Blood sampling
S: Stool sampling
U: Urine sampling

Table 4 List of study procedures (Amended: 08 February 2018)

Epoch	Screening	Vaccination						Challenge				
Type of contact	**Screening visit**	**Visit 1**	**Phone Call**	**Visit 2**	**Visit 3**	**Phone call**	**Visit 4**	**Visit 5**	**Inpatient stay**	**Visit 6**	**Visit 7**	**Visit 8**
Timepoints (days)	**Day -45 to Day -1**	**Day 1**	**Day 3 and Day 7**	**Day 8**	**Day 29**	**Day 31 and Day 35**	**Day 36**	**Day 57**	**Day 57 to Day 65 [8]**	**Day 85**	**Day 113**	**Day 237 (Month 8)**
Sampling timepoints	**Screening**	**Pre-vacc 1**		**Post-vacc 1**	**Pre-vacc 2**		**Post-vacc 2**	**Pre-challenge[9]**	**Inpatient stay**	**Post-challenge**		
Informed consent	●											
Check inclusion/exclusion criteria	●				●			●				
Collect demographic data	●											
Measure/record height and weight	●											
Medical history	●											
History directed physical examination				O			O		O	O	O	O
Physical examination	●	●			●			●				
Urine pregnancy test [1]	●	●			●			●				●
Check contraindications to vaccination	O	O			O							
Pre-vaccination/placebo/challenge agent administration body temperature		●			●			●				
Vaccine/placebo/challenge agent administration												
Study group and treatment number allocation		●										
Recording of administered treatment number		●			●							
Vaccine/placebo administration		●			●							
Challenge with *S. sonnei* 53G								●				
Laboratory assays												
Blood sampling for antibody response (~20 mL)		● [2, 3]		●	● [2]		●	● [2, 3]	● [4]	●		
Blood sampling for PBMC isolation (α4β7 plasmablasts response and transcriptomics) (~***50*** mL)		●		●			●		● [4]			
Blood sampling for hematology (~5 mL) [5]	●			●	●		●	●				●
Blood sampling for biochemical analysis (~5 mL)	●											

Epoch	Screening	Vaccination						Challenge				
Type of contact	**Screening visit**	**Visit 1**	**Phone Call**	**Visit 2**	**Visit 3**	**Phone call**	**Visit 4**	**Visit 5**	**Inpatient stay**	**Visit 6**	**Visit 7**	**Visit 8**
Timepoints (days)	**Day -45 to Day -1**	**Day 1**	**Day 3 and Day 7**	**Day 8**	**Day 29**	**Day 31 and Day 35**	**Day 36**	**Day 57**	**Day 57 to Day 65 [8]**	**Day 85**	**Day 113**	**Day 237 (Month 8)**
Sampling timepoints	**Screening**	**Pre-vacc 1**		**Post-vacc 1**	**Pre-vacc 2**		**Post-vacc 2**	**Pre-challenge[9]**	**Inpatient stay**	**Post-challenge**		
Serology for virology: HIV, HBV, HCV (~10 mL)	●											
Blood test for HLA-B27 and local anti *S. sonnei* LPS IgG (Screening-ELISA) (~15 mL)	●											
Urine sampling for urinalysis [6]	●							●				
Stool sample for sIgA		●		●			●		● [4]			
Stool sample for microbiome testing [7]		●						●				
Stool assessment: weight, consistency, blood and *S. sonnei* by culture and qPCR									●			
Safety assessment												
Record any concomitant medications/vaccinations	●	●		●	●		●	●	●	●	●	
Distribution of diary cards		O			O							
Return of diary cards				●			●					
Diary card transcription by investigator				●			●					
Recording of solicited adverse events (1–7 days post-vaccination)				●			●					
Recording of non-serious adverse events within 28 days post-vaccination				●	●		●	●				
Recording of AESI, SAEs and pregnancies	●	●		●	●		●	●	●	●	●	●
Recording of SAEs related to study participation, or to a concurrent GSK medication/vaccine	●	●		●	●		●	●	●	●	●	●
Study Conclusion												●

V: Visit, **D**: Day, **Vacc**: Vaccination; **HIV**: Human Immunodeficiency Virus, **HCV**: Hepatitis C Virus, **HBV**: Hepatitis B Virus, **ELISA**: Enzyme-linked immunosorbent assay, **AESI**: Adverse Events of Specific Interest, **SAEs**: Serious Adverse Events.

● is used to indicate a study procedure that requires documentation in the individual eCRF.

O is used to indicate a study procedure that does not require documentation in the individual eCRF.
[1] Female subjects will be randomized and study vaccine/placebo or challenge agent may only be administered if the pregnancy test is negative.
[2] Serum bactericidal assay will be performed at these visits in addition to the *S. sonnei* LPS IgG antibody response.
[3] Fc glycosylation test on leftover serum samples will be performed at these visits in addition to the *S. sonnei* LPS IgG antibody response and serum bactericidal assay.
[4] Sampling to be done 7 days after challenge agent administration (i.e., Day 64).
[5] Additionally, hematology is repeated weekly until resolution if neutropenia occurs. On the day of vaccination/challenge, sampling will be done before vaccination/challenge
[6] Urine dipstick to be done for all subjects and, in case of abnormal results, a urine culture is to be performed (urinalysis).
[7] Before vaccination/challenge (Day 1/Day 57 sample can be collected by the subject at most a week before and stored in a freezer).
[8] Sampling to be done daily during inpatient stay and if subject is still shedding 7 days post-challenge administration (Day 64). However, all subjects even if not shedding anymore will remain in the inpatient stay until 7 days post-challenge and will be discharged on Day 65.
[9] Samples collection can be performed between Day 56 and Day 57.

Whenever possible, the investigator should arrange study visits within the interval described in Table 5.

Table 5 Intervals between study visits

Interval	Optimal length of interval	Allowed interval
Screening visit → Visit 1	-7 days	-45 days – -1 days
Visit 1 → Phone Call	3 days	2 days - 6 days
Visit 1 → Visit 2	7 days	7 days - 10 days
Visit 1 → Visit 3	28 days	26 days - 33 days
Visit 3 → Phone call	3 days	2 days - 6 days
Visit 3 → Visit 4	7 days	7 days - 10 days
Visit 3 → Visit 5	28 days	26 days - 33 days
Visit 5 → Inpatient stay	9 days	9 days - as needed
Visit 5 → Visit 6	28 days	28 days - 35 days
Visit 5 → Visit 7	56 days	56 days - 70 days
Visit 5 → Visit 8	180 days	166 days - 194 days

3. OBJECTIVES

3.1. Primary objective

Efficacy

To demonstrate the efficacy of two vaccinations with 25 µg of 1790GAHB vaccine in healthy adults compared to placebo in reducing shigellosis, fulfilling the primary protocol case definition, after challenge with *S. sonnei* strain 53G.

Criterion:

Vaccine efficacy (VE) of 1790GAHB vaccine will be shown if the lower limit (LL) of the 90% confidence interval (CI) of VE will be above zero.

Refer to Section 4.1 below for the protocol case definition of shigellosis and to Section 4.2 for the definition of the primary endpoint.

3.2. Secondary objectives

Efficacy

- To determine the efficacy of the 1790GAHB vaccine compared to placebo against:
  - Shigellosis, as per definition requested by the Controlled Human Infection Model (CHIM) expert working group (see Section 6.3.3).
  - Shedding of *S. sonnei* strain 53G
  - Severe diarrhea.
  - More severe diarrhea.
  - Dysentery.
  - Weight of all grade 3-5 stools.
  - The total number of grade 3-5 stools.
  - Confirmed *S. sonnei* 53G shedding AND [moderate or severe diarrhea OR dysentery OR presence of fever ≥ 38.5°C OR presence of one or more severe intestinal symptoms (abdominal pain, cramping, nausea, vomiting …)].
  - Disease not fulfilling the protocol primary case definition for shigellosis associated or not with mild to moderate symptoms including: passing loose stool (not meeting the protocol definition of moderate or severe diarrhea), abdominal pain, abdominal cramps, gas, anorexia, nausea, headache, myalgia, malaise, arthralgia, fever, vomiting and IV fluid administration.
  - Time to onset of shigellosis after challenge

Safety

- To assess the safety and reactogenicity of the 1790GAHB vaccine in terms of solicited symptoms, unsolicited symptoms, serious adverse events (SAEs), adverse events of special interest (AESI) and laboratory parameters.
- To assess safety after challenge in terms of SAEs, AESI and laboratory parameters.

Immunogenicity

- To evaluate the IgG ELISA (Enzyme-linked immunosorbent assay) immunogenicity profile of the 1790GAHB vaccine at 7, and 28 days after the first and second vaccination (IgG ELISA coated with O antigen (OAg) containing Lipopolysaccharide (LPS)).
- To evaluate the immunogenicity profile of the 1790GAHB vaccine at pre-challenge and at 7 and 28 days after challenge (IgG ELISA coated with OAg containing LPS).
- To assess seroresponse for anti-*S. sonnei* LPS at 7, and 28 days after first and second vaccination.
- To assess post vaccination concentration ≥ 121 EU/ml for anti-LPS *S. sonnei* at 28 days after first and second vaccination

Refer to Section 4.3 for the definition of the secondary endpoints.

3.3. Tertiary objectives

The following tertiary objectives which are part of the study exploratory objectives may be evaluated in addition to the primary and secondary objectives and will complement assessment of the vaccine immunogenicity profile:

- Evaluate Secretory Immunoglobulin A (sIgA) in stool at Day 1 and 7 days after first and second vaccination and 7 days after challenge (Day 64).
- Evaluate presence and migration of α4β7 plasmablast at Day 1 and 7 days after first and second vaccination and 7 days after challenge (Day 64).
- Evaluate serum bactericidal activity against *S. sonnei* at Day 1 and 28 days after first and second vaccination.
- Evaluate correlation between serum anti-*S. sonnei* LPS IgG level, serum bactericidal activity titer, shigellosis, shedding, mild, moderate, severe or more severe diarrhea, and dysentery after challenge.
- Evaluate glycosylation of fragment crystallisable (Fc) portion of IgG antibodies at Day 1 and 28 days after second vaccination and association with clinical protection and/or serological endpoints.
- Evaluate gene expression signatures at Day 1 and 7 days after first and second vaccination and 7 days after challenge (Day 64).
- Investigate gut microbiome at Day 1 and 28 days after second vaccination and its potential association with protection and immune response.

These last two tertiary objectives involve pharmacogenomics testing. Refer to the Glossary of the protocol for the definition of Pharmacogenomics.

Refer to Section 4.4 for the definition of the tertiary endpoints. Details for the analysis of the tertiary objective will be reported in a please refer to a subsequent SAP.

4. ENDPOINTS

4.1. Case Definitions

Stools

Stools will be graded as follows:

- Grade 1: firm formed;
- Grade 2: soft formed;
- Grade 3: viscous opaque liquid or semi-liquid which assumes the shape of the bowl;
- Grade 4: watery opaque liquid;
- Grade 5: clear watery or mucoid liquid.

Diarrhea

For the purpose of this study, diarrhea is defined as:

- Moderate diarrhea: 4 to 5 loose or watery (Grade 3 to 5) stools or 400 to 800 grams of Grade 3 to 5 stools within 24 hours.
- Severe diarrhea: 6 or more loose or watery (Grade 3 to 5) stools or > 800 grams of Grades 3 to 5 stools within 24 hours or required medical intervention.
- More severe diarrhea: ≥ 10 loose or watery (Grade 3 to 5) stools or ≥ 1000 grams of Grade 3 to 5 stools within 24 hours or required medical intervention.
- Note: Diarrhea severity classes are not mutually exclusive, as more severe diarrhea is a subset of severe diarrhea.
- In case of severe diarrhea, medical intervention is defined as intravenous (IV) fluids administration or anticipation of antibiotic treatment before the 5th day after challenge.
- In case of more severe diarrhea, medical intervention is defined as ER visit or hospitalization for hypotensive shock.

Dysentery

For the purpose of this study, dysentery is defined as:

- A Grade 3, 4, or 5 stool with gross blood on at least 2 occasions within 24 hours AND presence of constitutional symptoms (i.e. fever, headache, fatigue, arthralgia, malaise, myalgia, chills, nausea, abdominal cramping, abdominal pain, gas, anorexia, vomiting).

Shigellosis

For the challenge phase of the study, the protocol primary case definition for shigellosis is:

- Shedding of *S.sonnei 53G* accompanied by moderate or severe diarrhea OR shedding with an ***oral temperature ≥ 38.5°C***.

4.2. Primary endpoint

Primary Efficacy endpoint

Vaccine efficacy (VE) will be evaluated in the 7-day post-challenge inpatient period after visit 5 (i.e. from challenge to discharge) by:

- Attack Rate of shigellosis (fulfilling the protocol case definition) occurring within a period starting with the challenge visit and lasting up to the end of the inpatient stay, in all subjects. The attack rate of shigellosis is expressed as proportion of subjects per group with at least one episode of shigellosis after challenge.

4.3. Secondary endpoints

Secondary Efficacy endpoints

All the secondary endpoints will be assessed in the 7-day post-challenge inpatient period after visit 5 (i.e. from challenge to discharge).

All subjects receiving the 1790GAHB vaccine vs. placebo will be compared according to the following endpoints:

a. Attack rate of shigellosis, with shigellosis defined by CHIM expert working group (see Section 6.3.3)

b. Proportion of subjects with shedding of *S. sonnei* strain 53G.

   Shedding of *S. sonnei* strain 53G is defined as positivity of at least one stool sample either by culture or quantitative Polymerase Chain Reaction (qPCR).

c. Proportion of subjects with at least one episode of severe diarrhea.

d. Proportion of subjects with at least one episode of more severe diarrhea.

e. Proportion of subjects with at least one episode of dysentery.

f. Mean (arithmetic or geometric, according to distribution of the data) weight of grade 3-5 stools per subject, for all subjects and for subjects with at least one grade 3-5 stool (i.e. grade 3-5 stool).

g. Mean weight per subject of all grade 3-5 stools accumulated from challenge to discharge

h. Mean number of grade 3-5 stools per subject from challenge to discharge.

i. Proportion of subjects with confirmed S. sonnei 53G shedding AND [moderate or severe diarrhea, OR dysentery, OR presence of fever (oral temperature ≥ 38.5°C), OR presence of at least one intestinal symptom (abdominal pain, abdominal cramping, gas, nausea, vomiting, anorexia) graded as severe].

j. Disease not fulfilling the protocol case definition for shigellosis associated or not with mild to moderate symptoms including:

    1. Proportion of subjects with at least one stool (not meeting the protocol definition of moderate or severe or more severe diarrhea),
    2. Proportion of subjects with at least one episode of abdominal pain,
    3. Proportion of subjects with at least one episode of abdominal cramps,
    4. Proportion of subjects with at least one episode of gas,
    5. Proportion of subjects with at least one episode of anorexia,
    6. Proportion of subjects with at least one episode of nausea,
    7. Proportion of subjects with at least one episode of headache,
    8. Proportion of subjects with at least one episode of myalgia,
    9. Proportion of subjects with at least one episode of malaise,
    10. Proportion of subjects with at least one episode of arthralgia,
    11. Proportion of subjects with at least one episode of fever (oral temperature ≥ 38.5°C),
    12. Proportion of subjects with at least one episode of vomiting, and
    13. Proportion of subjects with at least one IV fluid administration.

k. Time to onset of Shigellosis during the post-challenge period

All endpoints are measured from challenge to discharge.

Note that in the primary and secondary efficacy endpoints, fever is defined as oral temperature ≥ 38.5°C, in agreement with challenge model set up at Cincinnati. However, fever as solicited event will be defined as a body temperature of ≥38°C irrespective of route of measurement, in agreement with latest GSK standards and Brighton collaboration rules (see Section 6.5.2.2).

For derivation of the primary and secondary endpoints refer to Section 11.2.7.

Secondary Safety endpoints

The measures of safety will include:

- Number of subjects with solicited local and systemic reactions during 7 days following each vaccination. Solicited local reactions include injection site erythema, injection site induration and injection site pain; solicited systemic reactions include headache, arthralgia, chills, fatigue, malaise, myalgia, and fever (as measured orally).
- Number of subjects with reported unsolicited adverse events (of any nature and severity) during 28 days following vaccination and challenge, i.e. up to second vaccination visit (when evaluating after first vaccination) whichever comes first, and up to challenge visit (when evaluating after second vaccination) whichever comes first, and up to 28th day after challenge.
- Number of subjects with reported SAEs throughout the study duration.
- Number of subjects with reported AESI (i.e., symptomatic neutropenia) throughout the study duration.
- Number of subjects with deviations from normal values (i.e. reference range and/or clinically significant value, according to local ranges) of hematological tests at 7 days after first and second vaccination (Visit 2 and Visit 4) and at last study visit (Visit 8).

Secondary Immunogenicity endpoints

The measures of immunogenicity, against the LPS of *S. sonnei*, will include:

- IgG geometric mean concentrations (GMCs) pre-vaccination (Day 1), 7 days after first vaccination (Day 8), 28 days after first vaccination (Day 29), 7 days after second vaccination (Day 36), and 28 days after second vaccination (Day 57) overall and by antibody concentration at baseline (i.e., above vs. below the assay detection limit), as determined by anti-*S. sonnei* LPS IgG ELISA and applicable within-subject geometric mean ratios (GMRs) between post vaccination and baseline samples (D8/D1, D29/D1, D36/D1 and D57/D1).
- IgG GMC pre-challenge (Day 57), 7 and 28 days after challenge (Day 64 and Day 85) as determined by anti-*S. sonnei* LPS IgG ELISA and applicable within-subject geometric mean ratios (GMRs) between post-challenge and pre-challenge samples (D64/D57, and D85/D57).
- Number and percentage of seroresponders for anti-*S. sonnei* LPS at 28 days after first and second vaccination. Seroresponse is aimed to define a significant increase in anti *S. sonnei* LPS IgG concentration in post-vaccination samples and relies on the definition already used in a previous phase II study in Kenyan population. See Section 6.4.2 for definition of seroresponders.
- Number and percentage of subjects with titers post vaccination concentration ≥ 121 EU/ml for anti-LPS *S. sonnei* at 28 days after first and second vaccination

4.4. Tertiary endpoints

- Specific anti-S. sonnei LPS IgA antibody concentration in stool samples at Day 1, 7 days after first and second vaccination and 7 days after challenge (Day 64).
- Frequency of S. sonnei LPS specific IgG α4β7+ antibody secreting cells per 10^6 Peripheral Blood Mononuclear Cells (PBMC) plasmablast at Day 1 and 7 days after first and second vaccination and 7 days after challenge (Day 8, Day 36 and Day 64).
- Glycosylation profiles of IgG antibodies at Day 1 and 28 days after second vaccination (Day 57).
- Correlation between serum anti-S. sonnei LPS IgG concentration, Serum Bactericidal Assay (SBA) titer, shigellosis, shedding, Moderate to Severe Diarrhea (MSD), dysentery and mild diarrhea after challenge.
- The number of differential expressed genes 7 days after first and second vaccination and 7 days after challenge by comparing the relative abundance of mRNA sequences compared to Day 1 (pre-vaccination baseline).
- Diversity and frequency of microbiome components (taxa and/or genes according to technology) at Day 1 prior to vaccination and 28 days after second vaccination (Day 57).

Exploratory analysis for hypotheses generation will include the evaluation of the association of the microbiome components with efficacy and immunogenicity described above.

Additionally exploratory endpoints might be evaluated as per ICF. A further SAP will be provided for the analysis of the tertiary endpoints and reported in an Annex to the Clinical Study Report.

5. ANALYSIS SETS

5.1. Definition

5.1.1. All Enrolled Set

All screened subjects who provide informed consent, and provide demographic and/or baseline screening assessments and received a Subject ID, regardless of the subject's randomization and treatment status in the study.

5.1.2. All Exposed Set

All subjects in the enrolled set who receive a study vaccination. Please note that throughout Section 5, vaccine/study vaccination refer to study vaccine or placebo.

5.1.3. Safety Set

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

All subjects in the All Exposed Set with any valid data on solicited AEs.

5.1.3.2. Unsolicited Safety Set (unsolicited adverse events)

All subjects in the Exposed Set that do not withdraw early and are lost to follow-up on a vaccination visit.

5.1.3.3. Overall Safety Set

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

5.1.4. Full Analysis Set (FAS) for Efficacy/Immunogenicity

5.1.4.1. Full Analysis Set for Efficacy

All subjects in the All Enrolled Set who are randomized, receive a study vaccination and provide efficacy data.

5.1.4.2. Full Analysis Set for Immunogenicity

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

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).

5.1.5. Per-Protocol Set (PPS) for Efficacy/Immunogenicity

All subjects in the FAS for Efficacy/Immunogenicity who:

- Correctly receive the vaccines (i.e., receive the vaccine to which the subjects is randomized and at the scheduled timepoints).

Have no protocol deviations leading to exclusion as defined prior to unblinding / analysis.

Are not excluded due to other reasons defined prior to unblinding or analysis.

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.

The primary analysis will be based on the PPS for efficacy and immunogenicity. However, selected efficacy and immunogenicity secondary analyses will be performed on both FAS and PPS sets (more details in Section 6).

5.2. Criteria for eliminating data from Analysis Sets

Elimination codes are used to identify subjects to be eliminated from analysis. Detail is provided below for each set. Consolidated tables are also available in Section 12.

5.2.1. Elimination from Exposed Set (ES)

Code 1030 (Study vaccine not administered at all) and code 900 (invalid informed consent or fraud data) will be used for identifying subjects eliminated from ES.

5.2.2. Safety Set

Code 1150 (Subject did not provide any post-vaccination/post-challenge unsolicited safety data) will be used for identifying subjects eliminated from the Unsolicited Safety Set.

Code 1160 (Subject did not provide any post-vaccination solicited safety data) will be used for identifying subjects eliminated from the Solicited Safety Set.

Subjects with codes 2155 will be eliminated from the Overall Safety Set.

5.2.3. Elimination from Full Analysis Set (FAS) for Efficacy

5.2.3.1. Excluded subjects

A subject will be excluded from the FAS for Efficacy analysis under the following conditions

Code	Condition under which the code is used
900	Invalid informed consent or fraud data Signed informed consent not available on site Informed consent not signed and/or dated by subject Informed consent not signed prior to any study procedure Fraudulent Data
1030	Study vaccine not administered for both doses

3000.a	Did not undergo challenge
3000.c	Missed or incomplete assessment (after challenge)

5.2.4. Elimination from Per-protocol analysis Set (PPS) for Efficacy

5.2.4.1. Excluded subjects

A subject will be excluded from the PPS analysis for Efficacy under the following conditions

Code	Condition under which the code is used
900	Invalid informed consent or fraud data: Signed informed consent not available on site Informed consent not signed and/or dated by subject Informed consent not signed prior to any study procedure Fraudulent Data
1030	Study vaccine not administered for both doses
1040	Administration of concomitant vaccine(s) forbidden in the protocol*
1050	Randomization failure
1060	Randomization code was broken
1070	Vaccination not according to protocol: Incomplete treatment course Route of administration is wrong or unknown Treatment administered is correct but is not compatible with treatment regimen associated to treatment schedule Wrong replacement or study treatment administered Study treatment not prepared as per protocol (e.g. reconstitution) Site of injection for vaccine
1500	Incorrect Volume was given
1500	Study treatment not available at site for administration
1500	Study treatment administered while contraindication
1500	Pregnancy
1080	Vaccine temperature deviation
1090	Expired vaccine administered

2010	Protocol violation (inclusion/exclusion criteria)
2040	Administration of any medication forbidden by the protocol*
2050	medical condition forbidden
2060	infection related to the vaccine
2070	infection not related to the vaccine
2080	Subjects did not comply with vaccination schedule
2130	Subject not planned to be sampled for their all sampling visits: Testing performed on samples not aligned with ICF Collection of multiple samples when not required Subjects bled but not supposed to be bled Sample discard/destruction not completed as per protocol/SPM/ICF Sample destroyed and should not have been destroyed
3000.a	Did not undergo challenge
3000.b	Challenge administered out of the window
3000.c	Missed or incomplete assessment (after challenge)
3000.d	Challenge impacted by temperature excursion
3000.e	Challenge not prepared as per protocol (e.g. reconstitution)
3000.f	Challenge administered while contraindication

*See protocol Section 7.6.2 for specific details.

5.2.5. Elimination from Full Analysis Set (FAS) for Immunogenicity

5.2.5.1. Excluded subjects

A subject will be excluded from the FAS for Immunogenicity analysis under the following conditions

Code	Condition under which the code is used
900	Invalid informed consent or fraud data Signed informed consent not available on site Informed consent not signed and/or dated by subject Informed consent not signed prior to any study procedure Fraudulent Data
1030	Study vaccine not administered at all
2100	Serological results not available post-vaccination: Missed assessment (subject early terminated prior to the visit) Missed assessment (subject continues participation in the study) Low volume (not sufficient to perform testing) No samples collected for subject (and should have been collected) Mislabeling (sample not tested)
2120	Obvious incoherence or abnormality or error in data (for Antibody determination): Temperature deviations from range defined in protocol and/or SPM – refrigerator Temperature deviation from range defined in protocol and/or SPM - -20Freezer Temperature deviation from range defined in protocol and/or SPM - -45 freezer Temperature deviation from range defined in protocol and/or SPM - -80 freezer Central/internal/external lab deviations Mislabeling (sample tested)

*See protocol Section 5.3.

5.2.6. Elimination from Per-protocol analysis Set (PPS) for Immunogenicity

5.2.6.1. Excluded subjects

A subject will be excluded from the PPS analysis for Immunogenicity under the following conditions

Code	Condition under which the code is used
900	Invalid informed consent or fraud data Signed informed consent not available on site Informed consent not signed and/or dated by subject Informed consent not signed prior to any study procedure Fraudulent Data
1030	Study vaccine not administered
1040	Administration of concomitant vaccine(s) forbidden in the protocol*
1050	Randomization failure
1060	Randomization code was broken
1070	Vaccination not according to protocol: Incomplete treatment course Route of administration is wrong or unknown Treatment administered is correct but is not compatible with treatment regimen associated to treatment schedule Wrong replacement or study treatment administered Study treatment not prepared as per protocol (e.g. reconstitution) Site of injection for vaccine
1500	Incorrect Volume was given
1500	Study treatment not available at site for administration
1500	Study treatment administered while contraindication
1500	Pregnancy
1080	Vaccine temperature deviation
1090	Expired vaccine administered
2010	Protocol violation (inclusion/exclusion criteria)**
2040	Administration of any medication forbidden by the protocol*

2050	medical condition forbidden
2060	infection related to the vaccine
2070	infection not related to the vaccine
2080	Subjects did not comply with vaccination schedule
2090	Subjects did not comply with blood sample schedule
2100	Serological results not available post-vaccination: Missed assessment (subject early terminated prior to the visit) Missed assessment (subject continues participation in the study) Low volume (not sufficient to perform testing) No samples collected for subject (and should have been collected) mislabeling (sample not tested)
2120	Obvious incoherence or abnormality or error in data (for Antibody determination): Temperature deviations from range defined in protocol and/or SPM – refrigerator Temperature deviation from range defined in protocol and/or SPM - -20Freezer Temperature deviation from range defined in protocol and/or SPM - -45 freezer Temperature deviation from range defined in protocol and/or SPM - -80 freezer Central/internal/external lab deviations Mislabeling (sample tested)
2130	Subject not planned to be sampled: Testing performed on samples not aligned with ICF Collection of multiple samples when not required Subjects bled but not supposed to be bled Sample discard/destruction not completed as per protocol/SPM/ICF Sample destroyed and should not have been destroyed

*See protocol Section 7.6.2 for specific details.

**See protocol Section 5.3.

5.3. Important protocol deviation not leading to elimination from per-protocol analysis set

Important protocol deviations not leading to elimination from PPS and FAS will be defined in the Protocol Deviation Management Plan.

6. STATISTICAL ANALYSES

Note that standard data derivation rule and stat methods are described in Section 11 Annex 1 and will not be repeated below.

6.1. Demography

6.1.1. Analysis of demographics/baseline characteristics planned in the protocol

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

Distributions of subjects by gender and geographic ancestry will be summarized for the Exposed Set.

6.1.2. Additional considerations

Demography data will be tabulated also for the All Enrolled Set.

6.2. Exposure

6.2.1. Analysis of exposure planned in the protocol

Not applicable.

6.2.2. Additional considerations

The frequencies and percentages of subjects with vaccination will be summarized overall and by vaccine/placebo group. Data will be tabulated for the All Exposed Set.

6.3. Efficacy/Effectiveness

6.3.1. Analysis of efficacy planned in the protocol

VE will be evaluated at the end of study period. VE will be estimated as 1-RR where RR is the risk ratio (proportion of subjects reporting the disease in the vaccinated group over the proportion in the placebo group) together with 90% CIs.

Subjects with missing values of efficacy endpoints 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.

6.3.2. Additional considerations

All the efficacy objectives (i.e. primary and secondary) will be evaluated in the PPS.

For the primary efficacy endpoint, VE will be assessed as 1-RR, where RR is the ratio of proportion of subjects with shigellosis in the vaccinated group on the proportion of subjects with shigellosis in the placebo group. As per protocol definition, **vaccine efficacy in reduction of shigellosis** will be demonstrated if the lower limit of the 90% CI of VE is above zero (see Section 11.1 for methods of calculation of the 90% CI for VE).

The following calculation will be performed for all secondary efficacy endpoints, according to the nature of the data:

In case of endpoints measured as proportion (endpoints from a. to g., k., and l. sub-endpoints in Section 4.3), VE and 95% CI will be calculated as described above in the primary endpoint.

For count endpoint (i.e. total number of grade 3-5 stools) the RR will be estimated as the rate of grade 3-5 stools in vaccinated subjects divided by the rate of grade 3-5 stools in placebo. 95% CI for this rate ratio will be calculated from an unadjusted negative binomial model, in order to take into account possible over-dispersion of data (DIST=NB in GENMOD SAS procedure); a P-value from the associated two-tailed Wald will be considered statistically significant at alpha level of 0.05.

Continuous endpoint (i.e. weight of grade 3-5 stools per subjects, weight of grade 3-5 stools per subject in those with at least a grade 3-5 stools, and weight of grade 3-5 stools accumulated from challenge to discharge per subject) will be compared using unequal variance two-sample t-test in case of normality of data, unequal variance two-sample t-test on the log values in case log-values are normally distributed, or by Wilcoxon 2-sample test otherwise. In case of comparison via log-values, the geometric mean will be calculated as well as the arithmetic mean and associated 95% CI. In all cases a P-value from two-tailed test will be considered statistically significant at alpha level of 0.05.

Time to event endpoint (i.e. hours from challenge administration to onset of shigellosis) will be visually depicted by a Kaplan-Meier cumulative incidence curves, and compared using the Log-rank test, with P-value considered statistically significant at al alpha level of 0.05.

Sensitivity analysis

As sensitivity analyses, the analysis for primary efficacy objective (i.e. assessing reduction of shigellosis) and for secondary efficacy objective a. (i.e. assessing reduction of shigellosis, with shigellosis defined by CHIM expert working group consensus meeting) will be repeated:

Using FAS, instead of PPS.

Using a confidence level of 95%, instead of 90% on the PPS.

Subgroup analysis

The analysis for primary efficacy objective will be repeated for the two subgroups of subjects who were responders or non responders at the pre-challenge visit immunogenicity assessment.

6.3.3. Definition of shigellosis according to BMGF

After protocol approval, a specific definition of shigellosis proposed by the CHIM expert working group consensus meeting, different from the one defined as primary efficacy objective, was explicitly requested by the Bill and Melissa Gates Foundation (BMGF) and therefore added among the secondary efficacy objectives/endpoints.

BMGF shigellosis definition is the following:

Moderate diarrhea OR severe diarrhea OR dysentery

With moderate diarrhea, severe diarrhea, and dysentery specifically defined as per Table 6.

Table 6 BMGF Diarrhea and Dysentery definitions

Primary Endpoint	Definition
Severe Diarrhea	≥6 loose stools* in 24 hours OR >800 grams loose stools* in 24 hours
Moderate Diarrhea	[4-5 loose stools* in 24 hours OR 400-800 grams loose stools* in 24 hours] AND [oral temperature ≥38.0˚C OR ≥1 moderate constitutional/enteric symptom‡ OR ≥2 episodes of vomiting in 24 hours]
Dysentery	≥2 loose stools* with gross blood (hemoccult positive) in 24 hours AND [oral temperature ≥38.0˚C OR ≥1 moderate constitutional/enteric symptom OR ≥2 episodes of vomiting in 24 hours]

* grade 3 to 5 stools
‡Nausea, abdominal pain/cramping, myalgia/arthralgia, malaise

6.4. Immunogenicity

6.4.1. Analysis of immunogenicity planned in the protocol

Analysis of binary variables:

The number and percentages of subjects will be summarized. Two-sided 95% CIs for the percentages will be computed.

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.

Analysis of continuous variables:

Antibody concentration below the limit of detection in the respective assay will be set to half the limit for the purposes of analysis. The limit will be calculated as the average limit of detection for the assays done on Day 1 sera. The antibody concentrations/titers will be logarithmically transformed (base10) to fulfil the normal distribution assumption. GMCs will be calculated, with their associated two-sided 95% CIs, by exponentiating the corresponding log-transformed means and their 95% CI.

Additionally, within-subject geometric mean ratios (GMRs) will be computed for geometric mean concentrations at each time point after vaccination versus baseline (Day 1) and after challenge versus pre-challenge (Day 57). The GMRs and 95% CIs will be constructed by exponentiating the mean within-subject differences in log-transformed titers and the corresponding 95% CIs. GMR will be calculated only for subjects with valid baseline or pre-challenge valid immunogenicity results.

6.4.2. Additional considerations

The PPS will be the primary analysis set for the immunogenicity objectives; however, a subset of immunogenicity objectives will be evaluated also in the FAS.

The percentage of subjects with seroresponse by titer at baseline and with post vaccination antibody level ≥ 121 IgG units and associated two-sided 95% Clopper-Pearson CIs will be computed by vaccine group at each visit.

Unadjusted GMCs and associated two-sided 95% CIs will be computed for each group (i.e. vaccine and placebo) at each visit (i.e. Visit 1-Day 1, Visit 2-Day 8, Visit 3-Day 29, Visit 4-Day 36, Visit 5-Day 57, End of inpatient stay-Day 64, and Visit 6-Day 85). For each group, unadjusted GMCs and their 95% CIs will be obtained by exponentiating (base 10) the means and the lower and upper limits of the 95% CIs of the log-transformed concentrations (base 10). Additionally, within-subject unadjusted GMRs and associated two-sided 95% CIs will be computed for Day 8, Day 29, Day 36, and Day 57, respectively versus baseline and for Day 64 and Day 85 respectively versus Day 57 (pre-challenge).

For further details on standard business rule for GMC/GMR computation, see Section REF _Ref508290693 \r \h * MERGEFORMAT 11 Annex 1.*Reverse Cumulative Distribution Curves*

The distribution of antibody concentrations will be displayed using Reverse Cumulative Distribution Curves (RCDC) by visit (Day 1, Day 8, Day 29, Day 38, Day 57, Day 64, and Day 85) for groups of subjects receiving vaccine and placebo [i.e. a plot for each of the 7 time-point reporting two curves, one for vaccine group and one for placebo group].

RCDC will be produced to display also the cumulative weight of grade 3-5 stools and the number of grade 3-5 stools accumulated from challenge to discharge.

Derivation of immunogenicity data

a. Screening (anti *S. sonnei* LPS IgG Screening-ELISA)
   - The cut-off value for the anti *S. sonnei* LPS IgG Screening-ELISA is defined by the local laboratory.
   - A seronegative subject is a subject whose titer is below the cut-off value.
   - A seropositive subject is a subject whose titer is greater than or equal to the cut-off value.

b. Immunogenicity (anti S. sonnei LPS IgG-ELISA):
   - The cut-off value for the anti S. sonnei LPS IgG-ELISA is defined by the GVGH laboratory.
   - Subjects will be grouped in the following groups:
     - **Subjects with detectable antibodies at baseline** (i.e. with antibodies at baseline ≥ Lower Limit of quantification, LLOQ)
     - **Subjects without detectable antibodies at baseline** (i.e. with antibodies at baseline < LLOQ)

Please note the following difference: seronegative at baseline is evaluated at the screening visit by the center lab and it is a reason for exclusion; while detectable antibodies at baseline are evaluated by GVGH lab, and will determine subgroups to be analyzed.

All the immunogenicity analyses will be stratified by the above subgroups (i.e. presented overall, for subjects with detectable antibodies at baseline, and for subjects without detectable antibodies at baseline).

Seroresponse is aimed to define a significant increase in anti *S. sonnei* LPS IgG concentration in post-vaccination samples; it is defined in this study as follows:

- If the baseline value is greater than 50 EU/mL 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/mL then an increase of at least 25 EU/mL in the post-vaccination sample as compared to baseline [i.e. (Post-vac minus baseline) ≥ 25 EU].

Note: Threshold values/increases given in the definition of seroresponse might be subject to change during the course of the study (e.g., in case of optimization/qualification/validation of the anti *S. sonnei* LPS-IgG-ELISA).

6.5. Analysis of safety

6.5.1. Analysis of safety planned in the protocol

The primary analysis will be performed on the All Exposed Set.

Analysis of solicited local and systemic adverse events and other reactions

Solicited AEs will be summarized according to defined severity grading scales.

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

Post-vaccination/challenge solicited AE reported from Day 1 to 7 days post-vaccination/challenge will be summarized by maximal severity and by vaccine/placebo group. The severity of solicited local AE erythema and induration will be summarized according to categories based on linear measurement.

Injection site pain and systemic reactions occurring up to 7 days after each vaccination will be summarized according to “mild”, “moderate” or “severe”.

Each solicited local and systemic AE will also be further summarized as “none” versus “any”; same summary will be reported for all local together, all systemic together, and local and systemic.

Analysis of unsolicited AEs

All the AEs occurring during the study, judged either as probably related, possibly related, or not related to vaccination/challenge by the investigator, will be recorded.

The original verbatim terms used by investigators to identify AEs in the eCRFs will be mapped to preferred terms using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary. The AEs will then be grouped by MedDRA preferred terms into frequency tables according to system organ class and PT. All reported AEs, as well as AEs judged by the investigator as at least possibly related to study vaccine/placebo or challenge agent, will be summarized according to system organ class and preferred term within system organ class. These summaries will be presented by vaccination group. When an AE occurs more than once for a subject, the maximal severity and strongest relationship to the vaccine/placebo group will be counted.

Separate summaries will be produced for the following categories:

a. SAEs
b. AEs that are possibly or probably related to vaccine/placebo or challenge agent.
c. AEs leading to withdrawal from the study.
d. All AEs occurring within 28 days following administration of vaccination or challenge

Data listings of all AEs will be provided by subject.

In addition, AEs in the categories above will be provided as listed data.

6.5.2. Additional considerations

Standard data derivation procedures for safety is available in Section 11.2.5.

6.5.2.1. Exclusion of implausible solicited Adverse Event

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 7.

Table 7 Implausible Solicited Adverse Events

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

6.5.2.2. Solicited Adverse Events

All analyses will be based on the 'as treated' Solicited Safety Set (see Section 11.2.5).

Post-vaccination Solicited Adverse Events will be reported from 30 minutes until 7 days after vaccination using structured diary cards. The analyses of solicited adverse events will be done separately for 30 minutes, 4 hours, 6 hours, and after that, for each day between day 2 until day 7 included. In addition solicited adverse events ongoing after day 7 will be presented as unsolicited AE.

The list of Solicited Local and Systemic adverse events is reported in the Table 8.

Table 8 List of Local and Systemic solicited adverse events

Local adverse events
Pain at injection site Erythema at injection site Induration at injection site
Systemic adverse events
Arthralgia Chills Fatigue Malaise Myalgia Fever Headache

The maximum intensity of local injection site erythema/induration/fever will be scored at GSK Biologicals as in Table 9 and Table 10.

Table 9 Intensity grade for Erythema/Induration

	Erythema/Induration
0:	< 25 mm
1:	≥ 25 - ≤ 50 mm
2:	> 50 - ≤ 100 mm
3:	> 100 mm

Table 10 Intensity grade for fever

Fever			
Grade 0 Absent	**Grade 1 Mild**	**Grade 2 Moderate**	**Grade 3 Severe**
< 38.0°C	≥ 38.0°C – < 39.0°C	≥ 39.0°C – <40.0°C	≥ 40.0°C

A list of Solicited AE be assessed after vaccination or after challenge agent administration with associated intensity is reported in the Table 11. Note that the AEs marked with # will be evaluated after both vaccination and challenge.

Table 11 Intensity scales for solicited symptoms

Adverse Event	Intensity grade	Parameter
After vaccine/placebo administration		
Pain at injection site	0	None
	1	Mild: Any pain neither interfering with nor preventing normal every day activities.
	2	Moderate: Painful when limb is moved and interferes with every day activities
	3	Severe: Significant pain at rest. Prevents normal every day activities.
Erythema at injection site		Record greatest surface diameter in mm
Induration at injection site		Record greatest surface diameter in mm
Fever#*		Record temperature in °C/°F
Headache#	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	That prevents normal activity
Fatigue#	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	That prevents normal activity
Arthralgia#	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	That prevents normal activity
Malaise#	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	That prevents normal activity
Myalgia#	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	That prevents normal activity
Chills	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	That prevents normal activity
After challenge agent administration		
Nausea	0	Absent
	1	Mild or transient; maintains reasonable intake
	2	Moderate discomfort; intake decreased significantly; some activity limited
	3	No significant intake and requires medical intervention
	4	Hospitalization required
Abdominal cramping	0	Absent
	1	No interference with daily activities
	2	Some interference with daily activities not requiring medical intervention
	3	Prevents daily activities and requires medical intervention
	4	ER visit or hospitalization
Abdominal pain	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
Gas	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity

Adverse Event	Intensity grade	Parameter
Anorexia	0	Absent
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
Vomiting	0	Absent
	1	One episode within a 24-hour period
	2	2 episodes within a 24-hour period
	3	>2 episodes within a 24-hour period or requires medical intervention
	4	ER visit or hospitalization for hypotensive shock
Diarrhea**	0	Normal
	1	2-3 Grade 3-5 stools (loose or watery) or <400 g/Grade 3-5 (loose or watery) stools per 24 hours
	2	4-5 Grade 3-5 stools (loose or watery) or 400-800 g/ (loose or watery) Grade 3-5 stools per 24 hours
	3	6 or more Grade 3-5 stools (loose or watery) or >800 g/Grade 3-5 (loose or watery) stools per 24 hours or requires medical intervention
	4	≥ 10 loose stools (Grade 3 to 5) or ≥ 1000 grams of Grade 3 to 5 stools within 24 hours or ER visit or hospitalization for hypotensive shock

#Same grading is used for events present both post-vaccination and post-challenge.
*Fever is defined as temperature ≥ 38.0°C / 100.4°F. The preferred location for measuring temperature in this study will be the oral cavity.
**The end of a diarrheal episode occurs when a volunteer does not pass any Grade 3-5 stool within 24 hours.
ER: emergency room.

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

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 from 30 Min, 4 hours, 6 hours, and specifically for each day between day 2 through Day 7.
4. Solicited adverse events and indicators of solicited adverse events, occurrence of at least one event by category (local, systemic) and interval from 30 Min through 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 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.

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: Number of days with solicited adverse events

The number of days with the adverse event is defined irrespective of severity. This means at least 'mild' solicited adverse event that are assessed qualitatively and ≥ 25 mm for erythema and induration. If a solicited adverse event continues beyond day 7 the period after day 7 is added.

Level 5: 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" (≥ 25 mm for erythema and induration) for a subject if he/she reports greater than "none" 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 from 30 min to day 7.

6.5.2.3. Unsolicited Adverse Events

All the unsolicited adverse events occurring within 28 days following administration of each vaccination or occurring within 28 days following challenge administration, judged either as probably related, possibly related, or not related to vaccination by the investigator, will be recorded. 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 and PT. 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 9.1.4 of protocol for definition) will be analyzed, i.e., excluding those after a subject has given informed consent but before vaccination: those events will be listed with an appropriate flag.

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

- Onset between first vaccination and second vaccination.
- Onset between second vaccination and challenge administration.

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

During the course of this study, symptomatic neutropenia will be considered as AESIs.

Table 12 List of potential AESI to be followed during the study

Blood disorders
• Symptomatic neutropenia (all Grades)

Neutropenia is defined as decrease of neutrophil count asymptomatically or symptomatically. Available local laboratory ranges will be used to define neutropenia. This is completely diagnosed by laboratory testing for complete blood count. However only symptomatic neutropenia cases will be considered as AESI and reported as such.

The following grading will be used to classify neutropenia:

- Grade 1: 1800-1500 cells/mL.
- Grade 2: 1499-1000 cells/mL.
- Grade 3: 999-500 cells/mL.
- Grade 4: < 500 cells/mL.

In performing their assessment of symptomatic neutropenia cases, investigators are strongly advised to use Table 13 below.

Table 13 Evaluation of symptomatic neutropenia

Common presenting symptoms of neutropenia	Physical findings on examination of a subject with neutropenia
Low-grade fever	Fever
Sore mouth	Stomatitis
Odynophagia	Periodontal infection
Gingival pain and swelling	Cervical lymphadenopathy
Skin abscesses	Skin infection: The skin examination focuses on rashes, ulcers, or abscesses
Recurrent sinusitis and otitis	Splenomegaly
Symptoms of pneumonia (e.g., cough, dyspnea)	Associated petechial bleeding
Perirectal pain and irritation	Perirectal infection
Neutropenic sepsis	**Other according to Investigator's opinion**
Neutropenic infection	
Neutropenic colitis	
Other according to Investigator's opinion	

In order to facilitate the documentation of AESI in the eCRF, a list of MedDRA preferred terms (PTs) and PT codes corresponding to the above diagnoses will be available to investigators at study start.

When there is enough evidence to make any of the above diagnoses, the AE must be reported as AESI. Symptoms, signs or conditions which might (or might not) represent the above diagnoses, should be recorded and reported as AEs but not as AESI until the final or definitive diagnosis has been determined, and alternative diagnoses have been eliminated or shown to be less likely.

6.5.2.4. 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 as per the following codes:

Solicited symptom	Local/Systemic symptom	Lower level term code	Corresponding Lower level term decode
Erythema at injection site	local	10015150	Erythema
Induration at injection site	local	10060708	Induration
Pain at injection site	local	10022086	Injection site pain
Arthralgia	systemic	10003239	Arthralgia
Chills	systemic	10008531	Chills
Fatigue	systemic	10016256	Fatigue
Fever	systemic	10016558	Fever
Headache	systemic	10019211	Headache
Malaise	systemic	10025482	Malaise
Myalgia	systemic	10028411	Myalgia

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 preferred terms and according to occurrence of each event.

6.5.2.5. Clinical Safety Laboratory Investigations

The investigator must assess all safety laboratory results (see **Section 9.1.4** of the study protocol). Clinically significant modifications in hematology test values will be assessed by medical judgment based on interpretation of deviations from the institution's normal values. Abnormal laboratory findings (e.g., clinical chemistry, hematology, urinalysis) or other abnormal assessments that are judged by the investigator to be clinically significant will be recorded as AE or SAE if they meet the definition of an AE or SAE (refer to **Sections 9.1.1** and **9.1.2** of the Study Protocol).

Clinically significant abnormal laboratory findings or other abnormal assessments that are present at baseline and significantly worsen following the start of the study will also be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgement in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant. Clinically significant modifications in hematology will be assessed by medical judgment based on interpretation of deviations from institution's normal values and recommendations from CBER (FDA Center for Biologics Evaluation and Research) FDA GUIDANCE FOR INDUSTRY: Toxicity Grading Scale for Healthy Adults and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials and predefined list of sign and symptoms related to neutropenia.

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 (4 x 3 shift tables, including missing at baseline as a category).

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.

6.5.2.6. Concomitant Medication

Medications will be coded using the GSKDRUG dictionary.

The frequencies and percentages of subjects starting/reporting concomitant medications/products/vaccination during all study period will be tabulated by vaccine group for each study dose and across doses. See Section 7.6.1 of the Study Protocol for the list of medications/products/vaccination recorded in the eCRF.

An overview of the protocol-required reporting periods for AEs, SAEs, and pregnancies is given below.

Table 14 Reporting periods for collecting safety information

Timepoints	Scr	D1	D8	D29	D36	D57	D57-D64	D85	D237 (M8)
Solicited local and systemic AEs									
Unsolicited AEs									
AEs/SAEs leading to withdrawal from the study									
AESI SAEs									
Pregnancies									
SAEs related to study participation or concurrent GSK medication/vaccine									

Scr: Screening
D: Day
M: Month

7. ANALYSIS INTERPRETATION

Except for analyses on objectives with a pre-defined success criterion and an appropriate type I error control (see Section 3.1), comparative analyses will be descriptive with the aim to characterize the difference in reactogenicity and immunogenicity efficacy between groups. These descriptive analyses should not be interpreted.

8. CONDUCT OF ANALYSES

8.1. Sequence of analyses

Description	**Analysis ID**	**Disclosure Purpose (CTRS=public posting, SR=study report, internal)**	**Dry run review needed (Y/N)**	***Study Headline Summary (SHS)*requiring expedited communication to upper management (Yes/No)**	**Reference for TFL**
Interim Efficacy Analysis	E1_01	SR	Y	*Yes*	Tables 14.4.1.1 to 14.4.7 from 205626 (S SONNEI MONO GMMA SBVGH-003 [H03_03TP]) TOC
Final Analysis	E1_02	SR, CTSR	Y	No	205626 (S SONNEI MONO GMMA SBVGH-003 [H03_03TP]) TOC

The analysis for the Independent Data Monitoring Committee (IDMC) is planned to be performed by an Independent Data Analysis Center (IDAC). This analysis is planned as per IDMC Charter and will be provided three times, once after each of the first three cohorts (36 subjects expected) have completed the challenge phase of the trial.

One interim analysis is intended after the last subject of the last cohort had been discharged and will include all post-challenge efficacy objectives not requiring laboratory data on all subjects. In other words, all efficacy endpoints reported in Section 4.2 and 4.3 will be analyzed, but the positivity to shedding of *S. sonnei* 53G will be assessed only by culture results and not by qPCR.

An integrated clinical study report containing all data will be written and made available to the investigators after the final analysis.

If the data for tertiary endpoints become available at a later stage, (an) additional analysis/analyses will be performed. These data will be documented in annex(es) to the study report and will be made available to the investigators at that time.

8.2. Statistical considerations for interim analyses

No statistical adjustment will be made for the interim analyses. The need of the interim analysis on efficacy is that it serves as Go/No Go decision for start of the 4-component Shigella vaccine trial.

9. CHANGES FROM PLANNED ANALYSES

Below is reported a list of major changes from protocol Amendment 2 (22-Jun-2018) to this SAP. At the moment of SAP finalization, all the below changes are under implemented in the Protocol Amendment Form, and will be implemented in the upcoming Amendment 3 of the Protocol.

1. The BMGF requested a new shigellosis definition byCHIM (Controlled Human Infection Model) working group after protocol approval. It was added as secondary efficacy endpoint.
2. Additional efficacy endpoints had been added after late revision of the SAP by clinical team:
    a. Weight of grade 3-5 stool, instead of weight of grade 3-5 diarrhea episodes.
    b. Total number of grade 3-5 stool, instead of total number of grade 3-5 diarrhea episodes.
    c. Time to onset of shigellosis after challenge
3. Though data were collected, analysis of Immunogenicity at 7 days after each vaccine was missing, therefore it was added.
4. Analysis of percentage of subjects with immunogenicity $\geq$ 121 EU/ml after vaccination was added, as 121 EU is an historical value of a panel of 87 Israeli convalescent subjects after natural infection by S. Sonnei.
5. Medical interventions needed in case of “Severe Diarrhea” and “More Severe Diarrhea” had been clarified.
6. As it is of interest to explore whether the efficacy of vaccine differs in the groups of subjects with different pre-challenge antibody titers the following subgroup analysis was added:
    d. The analysis for the primary endpoints will be repeated for the two subgroups of subjects above and below the limit of antibody quantification at the pre-challenge visit.
7. The interim analysis of immunogenicity on a subset of subjects and visits was removed, as it is not necessary.
8. As requested by FDA, unsolicited events, are going to be collected also 28 days after challenge.

10. LIST OF FINAL REPORT TABLES, LISTINGS AND FIGURES

The TFL TOC provides the list of tables/listings and figures needed for the study report. It also identifies the tables eligible for each analyses and their role (synopsis, in-text, post-text, SHS, CTRS,…). Note that all TFL aimed to be included as post-text are noted as post-text even if these are tabulation of individual data such as listing of SAE. The post-text material contains all source material for the study report and accordingly a post-text table may be redundant with an in-text table.

- The TFL TOC is reported in the "205626 (S SONNEI MONO GMMA SBVGH-003 (H03_03TP)) TOC 09Aug2018". The latest GSK TOC and mock-up catalogues available in SDD were used PPD
- Standard mock-up tables are reported in "205626 (S SONNEI MONO GMMA SBVGH-003 [H03_03TP]) Mock-up catalogue 09Aug2018.docx"
- Specific ad hoc tables not present in the catalogue (i.e. all efficacy tables) are reported in "205626 (S SONNEI MONO GMMA SBVGH-003 [H03_03TP]) Mock-up customs 09Aug2018.xlsx"

Please note that the above TOC and mock-up are intended as a draft and will be finalized before Database freeze.

11. ANNEX 1 STANDARD DATA DERIVATION RULE AND STATISTICAL METHODS

11.1. Statistical Method References

The 90% CIs for RRs calculated in the efficacy analyses (and the correspondent 90% CI for VE, defined as 1-RR) will be calculated using the Miettinen-Nurminen method [Miettinen O., Nurminen M. Comparative analysis of two rates. Statistics in Medicine, 4, 213–226, 1985].

The exact two-sided 95% CIs for a proportion within a group will be the Clopper-Pearson exact CI [Clopper CJ, Pearson ES. The use of confidence or fiducial limits illustrated in the case of binomial. *Biometrika*. 1934;26:404-413].

11.2. Standard data derivation

The v15 GSK standard data derivation will be adopted. However, custom programming will be implemented if needed.

11.2.1. Date derivation

- SAS date of birth derived from a character date: In case day is missing, 15 is used. In case day & month are missing, 30June is used. This is also applicable to start date of Ae and medication.
- Onset day for an event (ae, medication, vaccination, …): The onset day is the number of days between the last study vaccination & the onset/start date of the event. This is 1 for an event starting on the same day as a vaccination. See SAS date derived in case the start date of the event is incomplete.
- Duration: Duration of an event is expressed in days. It is the number of days between the start & the stop dates + 1. Therefore duration is 1 day for an event starting & ending on the same day.
- Association of an event to the specific epoch: An adverse event belongs to Epoch 002, if the onset date is before and excluding Visit 5 or the last contact date (assuming last contact date is before Visit 5), whichever is coming first. An adverse event belongs to Epoch 003, if the onset date is subsequent to and including Visit 5 or the last contact date (assuming last contact date is at Visit 5 or at a subsequent date), whichever is coming first.

11.2.2. Dose number

- The study dose number is defined in reference to the number of study visits at which vaccination occurred. More specifically dose 1 refers to all vaccines administered at the first vaccination visit while dose 2 corresponds to all vaccinations administered at the second vaccination visit even if this is the first time a product is administered to the subject.
- Relative dose: the relative dose for an event (AE, medication, vaccination) is the most recent study dose given before an event. In case the event takes place on the day a study dose is given, the related dose will be that of the study dose, even if the event actually took place before vaccination,. Otherwise, if the event actually took place the same day of vaccination and before vaccination, the related dose will be that of the previously administered dose.
- The number of doses for a product is the number of times the product was administered to a subject.
- The incidence per dose is the number of vaccination visits at which an event was reported among all vaccination visits.

11.2.3. Demography

- Age: Age at the reference activity, computed as the number of days between the date of birth and the reference activity and converted in months (= days *12 /365.25) (keeping 1 decimal digit).
- Conversion of weight to kg

  The following conversion rule is used:

  - Weight in Kilogram= weight in Pounds / 2.2
  - Weight in Kilogram =weight in onces / 35.2

  The result is rounded to 2 decimals.

- Conversion of height to cm

  The following conversion rule is used:

  - Height in Centimetres = Height in Feet * 30.48
  - Height in Centimetres = Height in Inch * 2.54

  The result is rounded to the unit (ie no decimal).

- Conversion of temperature to °C

  The following conversion rule is used:

  - Temperature in °Celsius = ((Temperature in °Fahrenheit -32) *5)/9

  The result is rounded to 1 decimal.

11.2.4. Immunogenicity

- For a given subject and given immunogenicity measurement, missing or non-evaluable measurements will not be replaced. Therefore, an analysis will exclude subjects with missing or non-evaluable measurements.
- The Geometric Mean Concentrations (GMC) calculations are performed by taking the anti-log of the mean of the log titre transformations. Antibody titres below the cut-off of the assay will be given an arbitrary value of half the cut-off of the assay for the purpose of GMC calculation. Refer to Section 6.4.2 for details regarding cut-off definition and calculations.
- All CI computed will be two-sided 95% CI.

11.2.5. Safety

- For the analysis of solicited symptoms, missing or non-evaluable measurements will not be replaced. Therefore the analysis of the solicited symptoms based on the Exposed Set will include only vaccinated subjects for doses with documented safety data (i.e., symptom screen completed). This corresponds to the Solicited Safety Set defined in the protocol (Section 11.5.3.1). More specifically the following rules will be used:
  - Subjects who documented the absence of a solicited symptom after each of vaccine dose will be considered not having that symptom after that dose.
  - Subjects who documented the presence of a solicited symptom and fully or partially recorded daily measurement over the solicited period will be included in the summaries at that dose and classified according to their maximum observed daily recording over the solicited period.
  - Subjects who documented the presence of a solicited symptom after one dose without having recorded any daily measurement will be assigned to the lowest intensity category at that dose (i.e., 38.0°C for fever or grade 1 for other symptoms).
  - Doses without symptom sheets documented will be excluded.
- For analysis of unsolicited adverse events, such as serious adverse events or adverse events by primary MedDRA term, and for the analysis of concomitant medications, all vaccinated subjects will be considered. Subjects who did not report the event or the concomitant medication will be considered as subjects without the event or the concomitant medication respectively. This corresponds to the Solicited Safety Set defined in the protocol (Section 11.5.3.2).

Note that for all tables described in this section, the way the percentage of subjects will be derived will depend on the event analyzed (see table below for details). As a result, the N value will differ from one table to another.

Event	N used for deriving % per subject for Vaccination phase	N used for deriving % per dose for Vaccination phase
Concomitant vaccination	All subjects with study vaccine administered	All study visits with study vaccine administered
Solicited general symptom	All subjects with at least one solicited general symptom documented as either present or absent (i.e., symptom screen completed)	All study visits with study vaccine administered and with at least one solicited general symptom documented as either present or absent (i.e., symptom screen completed)
Solicited local symptom	All subjects with at least one solicited local symptom documented as either present or absent (i.e., symptom screen completed)	All study visits with study vaccine administered and with at least one solicited local symptom documented as either present or absent (i.e., symptom screen completed)
Unsolicited symptom	All subjects with study vaccine administered	All study visits with study vaccine administered
Concomitant medication	All subjects with study vaccine administered	All study visits with study vaccine administered

11.2.6. Number of decimals displayed:

The following decimal description from the decision rules will be used for the demography, efficacy, immunogenicity and safety/reactogenicity.

Display Table	Parameters	Number of decimal digits
Demographic characteristics	Mean, median age	1
Demographic characteristics	SD (age)	1
Immunogenicity	Ratio of GMT/C	2
Reactogenicity	Mean, Min, Q1, Median, Q3, Max for duration	1
All summaries	% of count, including LL & UL of CI	1
All summaries	% of difference, including LL & UL of CI	2
All summaries	p-value	3

11.2.7. Efficacy

Specific data derivation will be put in place to derive the primary and the secondary efficacy endpoints before Database freeze.

Stool sample assessment will be combined with solicited events after challenge to derive diarrhea and dysentery as defined in the protocol and as defined by BMGF after protocol was approved.

An overview of the algorithms to be used to derive all efficacy endpoints is reported in Table 15.

Table 15 Derivation of efficacy endpoints

Endpoint	Definition / Derivation
Primary Efficacy	
Shigellosis	[Shedding of S. sonnei strain 53G] AND
	[(Moderate diarrhea) OR
	(Severe diarrhea) OR
	(oral temperature ≥ 38.5°C)]
Derivation of Shigellosis	
Moderate diarrhea	[4 to 5 stools[1] within 24 hours] OR
	[400 to 800 grams of stools[1] within 24 hours] OR
	[intravenous (IV) fluids administration] OR
	[anticipation of antibiotic treatment before the 5th day after challenge]
Severe diarrhea	[≥ 6 stools[1] within 24 hours] OR
	[> 800 grams of stools[1] within 24 hours] OR
	[ER visit or emergency care for hypotensive shock]
Secondary Efficacy	
a. Shigellosis as per BMGF definition	[Moderate diarrhea] OR

Endpoint	Definition / Derivation
	[Severe diarrhea] OR
	[Dysentery]
Derivation of Shigellosis as per BMGF definition	
Moderate diarrhea	[(4 to 5 stools[1] within 24 hours) OR
	(400 to 800 grams of stools[1] within 24 hours)]
	AND[2]
	[(oral temperature ≥ 38.0˚C) OR
	(≥1 constitutional/enteric symptom[3] graded ≥2) OR
	[≥2 episodes of vomiting within 24 hours])
Severe diarrhea	[≥6 stools[1] within 24 hours] OR
	[800 grams loose stools[1] within 24 hours]
Dysentery	[≥2 stools[1] with gross blood (hemoccult positive) in 24 hours]
	AND[2]
	[oral temperature ≥38.0˚C OR
	(≥1 constitutional/enteric symptom[3] graded ≥2) OR
	(≥2 episodes of vomiting in 24 hours)]
b. Shedding of *S. sonnei* strain 53G	At least one stool[1] sample positive to *S. sonnei* strain 53G either by culture or qPCR
c. Severe diarrhea	Severe diarrhea as defined under primary endpoint
d. More severe diarrhea	[≥ 10 stools[1] within 24 hours] OR
	[≥ 1000 grams of stools[1] within 24 hours]
e. Dysentery	[≥2 stools[1] with gross blood (hemoccult positive) in 24 hours]
	AND[2]
	(≥1 constitutional symptom[4] graded ≥1)
f. weight of grade 3-5 stools	weight of each stool[1] during follow-up
g. weight of grade 3-5 stools accumulated from challenge to discharge	For each subject sum the weight of each stool[1] accumulated from challenge to discharge
h. number of grade 3-5 stools	For each subject count the number of stools[1] accumulated from challenge to discharge
i.	[At least one stool[1] sample positive to *S. sonnei* strain 53G either by culture or qPCR]
	AND[2]
	[(Moderate diarrhea) OR
	(Severe diarrhea) OR
	(Dysentery as defined in f.) OR
	(oral temperature ≥ 38.5°C) OR
	(≥1 intestinal symptom[5] graded ≥3)]
j.	
1	[At least one stool (whichever is the grading)]
	AND[2] NOT

Endpoint	Definition / Derivation
	[(Moderate diarrhea) OR
	(Severe diarrhea) OR
2	≥1 abdominal pain graded ≥1
3	≥1 abdominal cramps graded ≥1
4	≥1 abdominal gas graded ≥1
5	≥1 abdominal anorexia graded ≥1
6	≥1 abdominal nausea graded ≥1
7	≥1 abdominal headache graded ≥1
8	≥1 abdominal myalgia graded ≥1
9	≥1 abdominal malaise graded ≥1
10	≥1 abdominal arthralgia graded ≥1
11	≥1 oral temperature ≥ 38.5°C
12	≥1 vomit graded ≥1
13	≥1 IV fluid administration
k. Time to onset of Shigellosis during the post-challenge period	Shigellosis is defined as in primary objective. The day associated to the first shigellosis (e.g. day of the first stool that defined the 24 hours time-window that actually defined shigellosis) is the time of onset of Shigellosis.

[1]Considering only stools graded 3-5
[2]Where AND combines conditions occurring in the 7-day post-challenge inpatient period
[3]Where constitutional/enteric symptom are: Nausea, Abdominal pain/cramping, Myalgia/arthralgia, Malaise
[4]Where constitutional symptoms are: fever, headache, fatigue, arthralgia, malaise, myalgia, chills, nausea, abdominal cramping, abdominal pain, gas, anorexia, vomiting
[5]Where intestinal symptoms are: abdominal pain, abdominal cramping, gas, nausea, vomiting, anorexia

12. ANNEX 2: SUMMARY ON ELIMINATION CODES

Please refer to the exclusion matrix for a comprehensive list of exclusion codes among all the analysis sets.

13. ANNEX 3: STUDY SPECIFIC MOCK TFL

Please refer to "205626 (S SONNEI MONO GMMA SBVGH-003 [H03_03TP]) Mock-up customs 09Aug2018.xlsx" for the study specific mocks.

The data display, title and footnote are for illustration purpose and will be adapted to the study specificity as indicated in the TFL TOC. Note that there may be few changes between the study specific SAP mock TFL and the final TFLs as editorial/minor changes do not require a SAP amendment