Statistical Analysis Plan	
Title:	A staged Phase I/II observer-blind, randomised, controlled, multi-country study to evaluate the safety, reactogenicity, and immune responses to the GVGH altSonflex1-2-3 vaccine against *S. sonnei* and *S. flexneri*, serotypes 1b, 2a, and 3a, in adults in Europe (Stage 1) followed by age de-escalation from adults to children and infants, and dose-finding in infants in Africa (Stage 2)
eTrack study number and Abbreviated Title	212149 (SHIGELLA 4 GMMA GVGH-001 (H06_01TP))
Scope:	All analyses for the primary and secondary objectives of the study and part of tertiary objectives. The analysis details for remaining tertiary objectives are described in a separate Statistical Analysis Plan (SAP).
Date of Statistical Analysis Plan	Amendment 2 Final: 19 Mar 2024
APP 9000058193 Statistical Analysis Plan Template V5 (Effective date: 1July2020)	

TABLE OF CONTENTS

PAGE

LIST OF ABBREVIATIONS 6

1. DOCUMENT HISTORY 8

2. OBJECTIVES/ENDPOINTS 9

3. STUDY DESIGN 14

4. ANALYSIS SETS 19
   - 4.1. Definition 19
   - 4.2. Criteria for eliminating data from Analysis Sets 19
     - 4.2.1. Elimination from Enrolled Set 19
     - 4.2.2. Elimination from Exposed Set (ES) 19
     - 4.2.3. Elimination from Full Analysis Set (FAS) 19
     - 4.2.4. Elimination from Per Protocol Set (PPS) 20
       - 4.2.4.1. Excluded participants 20
     - 4.2.5. Elimination from Safety Sets 24
       - 4.2.5.1. Unsolicited Safety Set 24
       - 4.2.5.2. Solicited Safety Set 24

5. STATISTICAL ANALYSES 25
   - 5.1. Analysis of demography and baseline characteristics 25
     - 5.1.1. Analysis planned in the protocol 25
     - 5.1.2. Additional considerations 25
   - 5.2. Immunogenicity 26
     - 5.2.1. Analysis planned in the protocol 26
     - 5.2.2. Additional considerations 28
       - 5.2.2.1. Sensitivity analyses 32
       - 5.2.2.2. Quantitative Decision Making 32
   - 5.3. Analysis of safety and reactogenicity 33
     - 5.3.1. Analysis planned in the protocol 33
     - 5.3.2. Additional considerations 34
       - 5.3.2.1. Analysis of solicited events 34
       - 5.3.2.2. Analysis of unsolicited AEs and SAEs 35
       - 5.3.2.3. Analysis of solicited events and unsolicited AEs 35
       - 5.3.2.4. Other safety analyses 36
   - 5.4. Tertiary endpoints 36
     - 5.4.1. Analysis planned in the protocol 36
     - 5.4.2. Additional considerations 37

6. ANALYSIS INTERPRETATION 41

7. INTERIM ANALYSES 41
   - 7.1. Sequence of analyses 41
   - 7.2. Statistical considerations for interim analyses 42

8. CHANGES TO THE PROTOCOL DEFINED STATISTICAL ANALYSIS 42

9. NON-STANDARD DATA DERIVATION RULES AND STATISTICAL METHODS 43
9.1. Data derivation 43
9.2. Statistical method 49
9.2.1. Adjusted GMT or GMC ratios 49

10. ANNEXES 50
10.1. Business rules for standard data derivations and statistical methods 50
10.1.1. Attributing events to vaccine doses 50
10.1.2. Handling of missing data 50
10.1.2.1. Dates 50
10.1.2.2. Laboratory data 51
10.1.2.3. Daily recording of solicited events 51
10.1.2.3.1. Studies with paper diaries 51
10.1.2.4. Unsolicited adverse events 52
10.1.3. Data derivation 52
10.1.3.1. Age at first dose in days 52
10.1.3.2. Age at first dose in months 53
10.1.3.3. Age at first dose in years 53
10.1.3.4. Weight 53
10.1.3.5. Height 53
10.1.3.6. Body mass index (BMI) 53
10.1.3.7. Temperature 53
10.1.3.8. Numerical serology results 54
10.1.3.9. Geometric mean titres (GMTs) and concentrations (GMCs) 54
10.1.3.10. Onset day 54
10.1.3.11. Duration of events 54
10.1.3.12. Counting rules for combining solicited and unsolicited adverse events 54
10.1.3.13. Counting rules for occurrences of solicited events 55
10.1.4. Display of decimals 55
10.1.4.1. Percentages 55
10.1.4.2. Differences in percentages 55
10.1.4.3. Demographic/baseline characteristics statistics 55
10.1.4.4. Serological summary statistics 56
10.1.5. Statistical methodology 56
10.1.6. SAS code 56

11. REFERENCES 59

LIST OF TABLES

PAGE

Table 1 Primary, secondary and part of tertiary objectives/endpoints 9
Table 2 Study groups, intervention and blinding in Stage 1 17
Table 3 Study groups, intervention and blinding in Adults in Stage 2 17
Table 4 Study groups, intervention and blinding in Children in Stage 2 17
Table 5 Study groups, intervention and blinding in Infants in Stage 2 18
Table 6 Analysis Sets 19
Table 7 Definition of Serious Adverse Event (SAE) 45

LIST OF FIGURES

PAGE

Figure 1 Study design overview 14

Figure 2 Dose-response scenarios and statistical tests for identifying the preferred dose of the altSonflex1-2-3 vaccine 29

Figure 3 QDM Critical values and decisions 32

List of abbreviations

AE	Adverse event
ANCOVA	Analysis of Covariance
BMGF	Bill and Melinda Gates Foundation
CI	Confidence Interval
CSR	Clinical Study Report
eCRF	electronic Case Report Form
ELISA	Enzyme-linked immunosorbent assay
ES	Exposed Set
EU/mL	Enzyme linked immunosorbent assay Unit per millilitre
FAS	Full Analysis Set
GMC	Geometric mean antibody concentration
GMR	Geometric Mean Ratio
GMT	Geometric mean antibody titre
GSK	GlaxoSmithKline
GVGH	GSK Vaccines for Global Health
IC50	Inhibitory concentration 50%
IDAC	Independent Data Analysis Centre
IgG	Immunoglobulin G
IU/mL	International units per millilitre
LAR	Legally Acceptable Representative
LL	Lower Limit of the confidence interval
LLOQ	Lower Limit of Quantification
LPS	Lipopolysaccharide
MedDRA	Medical Dictionary for Regulatory Activities
MV	Minimum Value
NA	Not Applicable
NIBSC	National Institute for Biological Standards and Control
OAg	O-Antigen
PD	Protocol Deviation
PPS	Per-Protocol Set
QDM	Quantitative Decision Making
SAE	Serious adverse event

SAP	Statistical Analysis Plan
SBIR	GSK Biologicals Internet Randomisation System
SD	Standard Deviation
SDTM	Study Data Tabulation Model
TAU	Tel Aviv University
TV	Target Value
UL	Upper Limit of the confidence interval
WRAIR	Walter Reed Army Institute of Research

1. DOCUMENT HISTORY

Date	Description	Protocol Version
21 SEP 2021	Final	Final: 26 MAY 2021
18 FEB 2023	Amendment 1 (alignment to protocol amendment and other minor changes)	Amendment 2, dated 14 FEB 2022
19 Mar 2024	Amendment 2 (alignment to protocol amendment 4 and inclusion of more tertiary objectives)	Amendment 4, dated 31 OCT 2023

Section #	Description of Amendment 2 change	Brief rationale
Section 2, Section 5.2	Reference ELISA has been replaced by GVGH ELISA for the secondary immunogenicity objectives/endpoints. LPS/OAg has been added consistently to all the endpoints using GVGH ELISA. GMTs have been replaced by GMCs.	Align to Protocol Amendment 3
Section 2	Wording has been added to indicate that endpoints measuring GVGH ELISA level equivalent 1:800 and 1:1600 thresholds are not validated thresholds for a correlate of protection against shigellosis. New reference added.	Align to Protocol Amendment 3 and new evidence
Section 2, Section 5.4	Additional tertiary endpoints for SBA have been added to evaluate subsets of all safety cohorts of Stage 2 participants and all infants in the dose finding cohort.	Align to Protocol Amendment 3
Section 2. Section 5.4	Replacement of reference ELISA testing of Stage 1 samples with testing of Stage 2 samples from adults, children and infants in safety cohorts up to 28 days after each vaccination; addition of corresponding endpoints.	Align to Protocol Amendment 4
Section 7.1	An interim analysis has been added after the second vaccination (Day 113) in the infant safety cohort.	Align to Protocol Amendment 3
Section 2, Section 5.4.1, Section 5.4.2	Inclusion of additional tertiary endpoints in scope for interim/final analysis, such as heterologous SBA, anti-protein ELISA and reference ELISA and their LLOQ cut off values, when known.	Tertiary endpoints results may become available in time of interim/final analysis complementing the primary read-out. Align to Protocol Amendment 4
Section 5.4.2	Correlation analysis between GVGH ELISA and SBA.	The vaccine will be better characterised by this assessment
Section 4	Elimination code for screen failures has been added.	As per data collection, a flag should be created to exclude screen failures from analysis sets.
Section 5.2.1	A clarification has been added that in case of a change in the benefit-risk profile due to a safety concern, a dose level may be discontinued.	Align to Protocol Amendment 3
Section 9.1	Modification of the creatinine grading scale of Stage 2 Infants to better reflect the population being evaluated.	Align to Protocol Amendment 4
Section 3	Replacement of INFANRIX with INFANRIX HEXA as comparator vaccine.	Align to Protocol Amendment 4
Entire document	Clarifications added.	To optimise document quality and align with TFL

2. OBJECTIVES/ENDPOINTS

Table 1 Primary, secondary and part of tertiary objectives/endpoints

Objectives	Endpoints
Primary	
• To identify the preferred dose of each component of the altSonflex1-2-3 vaccine (low, medium, or high) for infants 9 months of age in Africa (Stage 2).	• Anti-serotype specific *Shigella* lipopolysaccharide (LPS)/O-Antigen (OAg)* serum immunoglobulin G (IgG) geometric mean concentrations (GMC), as measured by GVGH enzyme-linked immunosorbent assay (ELISA), in infants 9 months of age in Africa, 28 days after the third vaccination (Day 281). **S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested.
• To evaluate the safety and reactogenicity of the altSonflex1-2-3 vaccine in all participants in Europe and Africa (Stage 1 and Stage 2).	• Number of adults 18 to 50 years of age in Europe with solicited administration-site events during 7 days after each vaccination (study intervention administered on Day 1 and Day 85/Day 169). • Number of adults 18 to 50 years of age in Europe with solicited systemic events during 7 days after each vaccination (study intervention administered on Day 1 and Day 85/Day 169). • Number of adults 18 to 50 years of age in Europe with unsolicited adverse events (AEs) during 28 days after each vaccination (study intervention administered on Day 1 and Day 85/Day 169). • Number of adults 18 to 50 years of age in Europe with serious adverse events (SAEs) during their entire study participation period (from Day 1 to Day113/Day 197). • Number of adults 18 to 50 years of age in Europe with deviations from normal values of haematological, renal, and hepatic panel test results, at 7 days after each vaccination (Day 8 and Day 92/Day 176). • Number of adults 18 to 50 years of age in Africa with solicited administration-site events during 7 days after each vaccination (study intervention administered on Day 1 and Day 85). • Number of adults 18 to 50 years of age in Africa with solicited systemic events during 7 days after each vaccination (study intervention administered on Day 1 and Day 85). • Number of adults 18 to 50 years of age in Africa with unsolicited AEs during 28 days after each vaccination (study intervention administered on Day 1 and Day 85). • Number of adults 18 to 50 years of age in Africa with SAEs during their entire study participation period (from Day 1 to Day 113). • Number of adults 18 to 50 years of age in Africa with deviations from normal values of haematological, renal, and hepatic panel test results, at 7 days after each vaccination (Day 8 and Day 92). • Number of children 24 to 59 months of age in Africa with solicited administration-site events during 7 days after each vaccination (study intervention administered on Day 1 and Day 85). • Number of children 24 to 59 months of age in Africa with solicited systemic events during 7 days after each vaccination (study intervention administered on Day 1 and Day 85). • Number of children 24 to 59 months of age in Africa with unsolicited AEs during 28 days after each vaccination (study intervention administered on Day 1 and Day 85).

Objectives	Endpoints
	• Number of children 24 to 59 months of age in Africa with SAEs during their entire study participation period (from Day 1 to Day 113). • Number of children 24 to 59 months of age in Africa with deviations from normal values of haematological, renal, and hepatic panel test results, at 7 days after each vaccination (Day 8 and Day 92). • Number of infants 9 months of age in Africa with solicited administration-site events during 7 days after each vaccination (study intervention administered on Day 1, Day 85, and Day 253). • Number of infants 9 months of age in Africa with solicited systemic events during 7 days after each vaccination (study intervention administered on Day 1, Day 85, and Day 253). • Number of infants 9 months of age in Africa with unsolicited AEs during 28 days after each vaccination (study intervention administered on Day 1, Day 85, and Day 253). • Number of infants 9 months of age in Africa with SAEs during their entire study participation period (from Day 1 to Day 281). • Number of infants 9 months of age in Africa with deviations from normal values of haematological, renal, and hepatic panel test results, at 7 days after each vaccination (Day 8, Day 92, and Day 260).
Secondary	
• To evaluate the immunogenicity profile of the altSonflex1-2-3 vaccine in all participants in Europe (Stage 1).	• Anti-serotype specific *Shigella* LPS/O-Antigen (OAg)* serum IgG geometric mean concentrations (GMCs), in adults 18 to 50 years of age in Europe, as measured by GVGH ELISA, before each vaccination (Day 1 and Day 85/Day 169), 14 days after the first vaccination (Day 15) and 28 days after each vaccination (Day 29 and Day 113/Day197). **S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested.
• To evaluate the immunogenicity profile of the altSonflex1-2-3 vaccine in all participants in Africa (Stage 2).	• Anti-serotype specific *Shigella* LPS/OAg* serum IgG GMCs, in adults 18 to 50 years of age in Africa, as measured by GVGH ELISA, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113). • Anti- serotype specific *Shigella* LPS/OAg* serum IgG GMCs, in children 24 to 59 months of age in Africa, as measured by GVGH ELISA, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113). • Anti-serotype specific *Shigella* LPS/OAg* serum IgG GMCs, in infants 9 months of age in Africa, as measured by GVGH ELISA, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281). **S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested.
• To further characterise the immunogenicity profile of the *S. sonnei* component of altSonflex1-2-3 vaccine in all participants in Europe and Africa (Stage 1 and Stage 2).	• Number of adults in Europe 18 to 50 years of age achieving a GVGH ELISA level equivalent to ≥1:800* titre against *S. sonnei* LPS/OAg, before each vaccination (Day 1 and Day 85/Day 169), 14 days after first vaccination (Day 15) and 28 days after each vaccination (Day 29 and Day 113/Day 197). • Number of adults in Africa 18 to 50 years of age achieving a GVGH ELISA level equivalent to ≥1:800* titre against *S. sonnei*

Objectives	Endpoints
	LPS/OAg, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113). • Number of children 24 to 59 months of age in Africa achieving a GVGH ELISA level equivalent to ≥1:800* titre against *S. sonnei* LPS/OAg, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113). • Number of infants 9 months of age in Africa achieving a GVGH ELISA level equivalent to ≥1:800* titre against *S. sonnei* LPS/OAg, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281). • Number of adults in Europe 18 to 50 years of age achieving a GVGH ELISA level equivalent to ≥1:1600** titre against *S. sonnei* LPS/OAg, before each vaccination (Day 1 and Day 85/Day 169), 14 days after first vaccination (Day 15) and 28 days after each vaccination (Day 29 and Day 113/Day 197). • Number of adults in Africa 18 to 50 years of age achieving a GVGH ELISA level equivalent to ≥1:1600** titre against *S. sonnei* LPS/OAg, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113). • Number of children 24 to 59 months of age in Africa achieving a GVGH ELISA level equivalent to ≥1:1600** titre against *S. sonnei* LPS/OAg in, before each vaccination (Day 1 and Day 85) and 28 days after each vaccination (Day 29 and Day 113). • Number of infants 9 months of age in Africa achieving a GVGH ELISA level equivalent to ≥1:1600** titre against *S. sonnei* LPS/OAg, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281). *This threshold (1:800, known as "Cohen's Threshold") is not validated [Conti, 2024] but has been previously reported to be potentially protective for *Shigella* [Cohen, 1988; Launay, 2017]. It will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the Statistical Analysis Plan (SAP). **This non-validated threshold (1:1600, double of "Cohen's Threshold") will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP.
• To evaluate seroresponse with the altSonflex1-2-3 vaccine after each vaccination in all participants in Europe and Africa (Stage 1 and Stage 2).	• Number of adults 18 to 50 years of age in Europe showing at least a 4-fold increase in anti-serotype specific *Shigella* LPS/OAg* serum IgG concentrations, as measured by GVGH ELISA, at 14 days after first vaccination (Day 15) and at 28 days after each vaccination (Day 29 and Day 113/Day 197), compared to baseline (Day 1 and Day 85/Day 169). • Number of adults 18 to 50 years of age in Africa showing at least a 4-fold increase in anti-serotype specific *Shigella* LPS/OAg* serum IgG concentrations, as measured by GVGH ELISA, at 28 days after each vaccination (Day 29 and Day 113), compared to baseline (Day 1 and Day 85). • Number of children 24 to 59 months of age showing at least a 4-fold increase in anti-serotype specific *Shigella* LPS/OAg* serum IgG concentrations, as measured by GVGH ELISA, at 28 days after each vaccination (Day 29 and Day 113), compared to baseline (Day 1 and Day 85).

Objectives	Endpoints
	• Number of infants 9 months of age showing at least a 4-fold increase in anti-serotype specific *Shigella* LPS/OAg* serum IgG concentrations, as measured by GVGH ELISA, at 28 days after each vaccination (Day 29, Day 113, and Day 281), compared to baseline (Day 1, Day 85, and Day 253). **S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested.
• To evaluate the immunogenicity of co-administered MR-VAC in infants 9 months of age in Africa (Stage 2), in the dose-finding cohort.	• Anti-measles IgG concentrations, as measured by ELISA, in infants 9 months of age in Africa, in the dose-finding cohort, before the first MR-VAC (Day 1) and at 28 days after the second MR-VAC vaccination (Day 281). • Anti-rubella IgG concentrations, as measured by ELISA, in infants 9 months of age in Africa, in the dose-finding cohort, before the first MR-VAC (Day 1) and at 28 days after the second MR-VAC vaccination (Day 281). • Number of infants 9 months of age, in the dose-finding cohort, achieving anti-measles IgG concentrations of ≥150 mIU/mL* and ≥ 200 mIU/mL*, as measured by ELISA, at 28 days after the second MR-VAC vaccination (Day 281). • Number of infants 9 months of age, in the dose-finding cohort, achieving anti-rubella IgG concentrations of ≥4 IU/mL* and ≥10 IU/mL*, as measured by ELISA, at 28 days after the second MR-VAC vaccination (Day 281). *This threshold is based on the vaccine correlate of protection and World Health Organization guidelines.
Tertiary	
• To evaluate the immune response induced by the altSonflex1-2-3 vaccine in 25% of adults in Europe (Stage 1), subsets of adults, children, and infants in the safety cohorts, and all infants in the dose-finding cohort, in Africa (Stage 2), using the serum bactericidal assay (SBA).	• Anti-serotype specific* serum bactericidal antibody geometric meant titres (GMTs) in 25% of adults in Europe 18 to 50 years of age, before each vaccination (Day 1 and Day 85/Day 169), 14 days after the first vaccination (Day 15) and 28 days after each vaccination (Day 29 and Day 113/Day197). • Anti-serotype specific* serum bactericidal antibody GMTs in a subset of adults in Africa 18 to 50 years of age, before the first vaccination (Day 1) and 28 days after each vaccination (Day 29 and Day 113). • Anti-serotype specific* serum bactericidal antibody GMTs in a subset of children 24 to 59 months of age before the first vaccination (Day 1) and 28 days after each vaccination (Day 29 and Day 113). • Anti-serotype specific* serum bactericidal antibody GMTs in a subset of infants 9 months of age in the safety cohort, before the first vaccination (Day 1) and 28 days after each vaccination (Day 29, Day 113, and Day 281). • Anti-serotype specific* serum bactericidal antibody GMTs in ***all*** infants 9 months of age in the dose-finding cohort, before each vaccination (Day 1, Day 85, and Day 253) and 28 days after each vaccination (Day 29, Day 113, and Day 281). **S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested.

Objectives	Endpoints
• To further explore immune responses to the altSonflex1-2-3 vaccine in 25% of adults in Europe (Stage 1) and a subset of infants 9 months of age in Africa (Stage 2), in the dose-finding cohort.	• Anti-serotype serum bactericidal antibody GMTs against a panel of vaccine heterologous *Shigella* serotypes, as measured by SBA. • Anti-serotype serum bactericidal antibody GMTs against a panel of vaccine homologous and heterologous *Shigella* serotypes, as measured by opsonophagocytosis assay.
• To measure natural exposure to *Shigella* in Stage 2 ***infant*** participants	• Anti-*Shigella*-conserved protein IgG, as measured by anti-protein ELISA.
• To evaluate the immunogenicity profile of the altSonflex1-2-3 vaccine as measured by reference ELISA in ***adult, child, and infant safety cohorts,*** in ***Africa (Stage 2)***.	• Anti-serotype specific *Shigella* LPS* serum IgG GMTs, in adults 18 to 50 years of age in ***Africa***, as measured by reference ELISA, before each vaccination (Day 1 and ***Day 85),*** and 28 days after each vaccination (Day 29 and ***Day 113)***. • ***Anti-serotype specific Shigella LPS* serum IgG GMTs, in children 24 to 59 months of age in Africa, as measured by reference ELISA, before each vaccination (Day 1 and Day 85), and 28 days after each vaccination (Day 29 and Day 113).*** • ***Anti-serotype specific Shigella LPS* serum IgG GMTs, in infants 9 months of age in the safety cohorts in Africa, as measured by reference ELISA, before 1st vaccination (Day 1), and 28 days after each vaccination (Day 29, Day 113, and Day 281).*** • Number of adults in ***Africa*** 18 to 50 years of age achieving a reference ELISA level equivalent to ≥1:800** titre against *S. sonnei* LPS, before each vaccination (Day 1 and ***Day 85),*** and 28 days after each vaccination (Day 29 and ***Day 113).*** • ***Number of children 24 to 59 months of age in Africa achieving a reference ELISA level equivalent to ≥1:800** titre against S. sonnei LPS, before each vaccination (Day 1 and Day 85), and 28 days after each vaccination (Day 29 and Day 113).*** • ***Number of infants 9 months of age in the safety cohorts in Africa achieving a reference ELISA level equivalent to ≥1:800** titre against S. sonnei LPS, before 1st vaccination (Day 1), and 28 days after each vaccination (Day 29, Day 113, and Day 281).*** • Number of adults in ***Africa*** 18 to 50 years of age achieving a reference ELISA level equivalent to ≥1:1600*** titre against *S. sonnei* LPS, before each vaccination (Day 1 and ***Day 85),*** and 28 days after each vaccination (Day 29 and ***Day 113)***. • ***Number of children 24 to 59 months of age in Africa achieving a reference ELISA level equivalent to ≥1:1600*** titre against S. sonnei LPS, before each vaccination (Day 1 and Day 85), and 28 days after each vaccination (Day 29 and Day 113).*** • ***Number of infants 9 months of age in the safety cohorts in Africa achieving a reference ELISA level equivalent to ≥1:1600*** titre against S. sonnei LPS, before 1st vaccination (Day 1), and 28 days after each vaccination (Day 29, Day 113, and Day 281).*** *S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a serotypes will be tested. **This threshold (1:800, known as "Cohen's Threshold") is not validated [Conti, 2024] but has been previously reported to be potentially

Objectives	Endpoints
	protective for *Shigella* [Cohen, 1988; Launay, 2017]. It will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP. ***This non-validated threshold (1:1600, double of "Cohen's Threshold") will be calibrated for the assay to be used in this study. The details of the equivalent threshold to be used will be reported in the SAP.

3. STUDY DESIGN

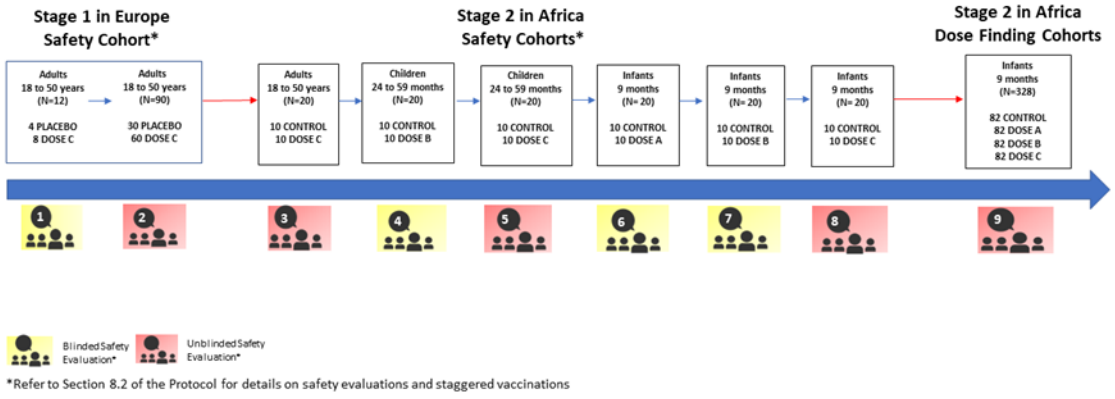
This is a Phase I/II observer-blind, controlled, self-contained, randomised, multi-country, staged, age-de-escalation study. An electronic Case Report Form (eCRF) and a Paper Diary (pDiary) will be used for data collection, which will include biological sampling (blood) and safety follow-ups.

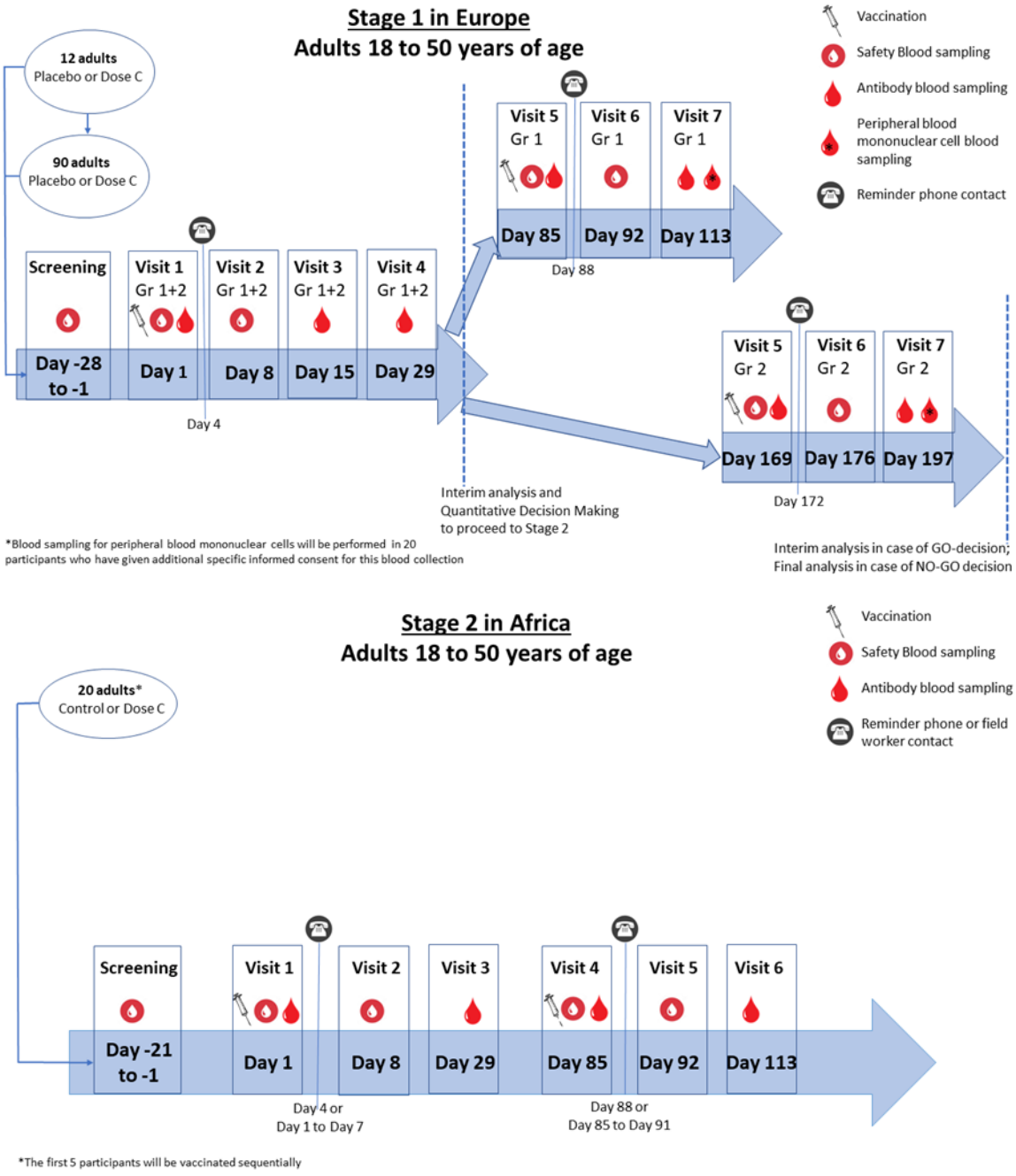
The study has 2 adult safety cohorts (18 to 50 years age in Europe and Africa), 2 child safety cohorts (24 to 59 months of age in Africa), 3 infant safety cohorts (9 months of age in Africa), and 1 infant dose-finding cohort (9 months of age in Africa). Figure 1 shows a visual representation of the study design. The study has a total of 20 study intervention groups as shown in Table 2, Table 3, Table 4, and Table 5 for Stage 1 adults, Stage 2 adults, Stage 2 children, and Stage 2 infants, respectively.

The study design, the enrolment, and the monitoring procedures will follow GSK safety standards for FTIH clinical studies (refer to Protocol Sections 8.2.2 and 10.1.5 for details on staggered vaccination, safety monitoring, and holding rules). For the study to proceed to Stage 2 in Africa, in addition to a favourable safety evaluation, a comprehensive review of Stage 1 data will also be performed with the support of a Quantitative Decision Making (QDM) approach (refer to SAP Section 5.2.2.2). In case, a No-Go decision is made, the study will be discontinued early and completed at the end of Stage 1.

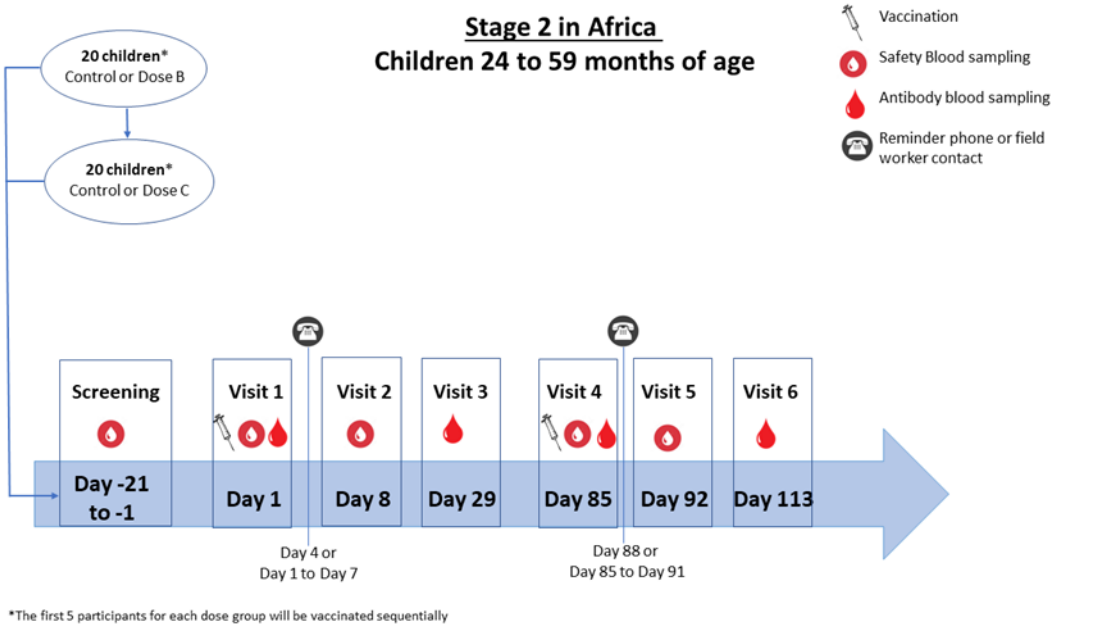
The primary completion date will be at Visit 9 (Day 281) in the Stage 2 infant dose-finding cohort.

Figure 1 Study design overview




Stage 1 in Europe
Adults 18 to 50 years of age
12 adults
Placebo or Dose C
90 adults
Placebo or Dose C
Screening
Visit 1
Gr 1+2
Visit 2
Gr 1+2
Visit 3
Gr 1+2
Visit 4
Gr 1+2
Day -28 to -1
Day 1
Day 8
Day 15
Day 29
Day 4
Visit 5
Gr 1
Visit 6
Gr 1
Visit 7
Gr 1
Day 85
Day 92
Day 113
Day 88
Visit 5
Gr 2
Visit 6
Gr 2
Visit 7
Gr 2
Day 169
Day 176
Day 197
Day 172
Interim analysis and
Quantitative Decision Making
to proceed to Stage 2
Interim analysis in case of GO-decision;
Final analysis in case of NO-GO decision
Vaccination
Safety Blood sampling
Antibody blood sampling
Peripheral blood mononuclear cell blood sampling
Reminder phone contact
*Blood sampling for peripheral blood mononuclear cells will be performed in 20 participants who have given additional specific informed consent for this blood collection

Stage 2 in Africa
Adults 18 to 50 years of age
20 adults*
Control or Dose C
Screening
Visit 1
Visit 2
Visit 3
Visit 4
Visit 5
Visit 6
Day -21 to -1
Day 1
Day 8
Day 29
Day 85
Day 92
Day 113
Day 4 or
Day 1 to Day 7
Day 88 or
Day 85 to Day 91
Vaccination
Safety Blood sampling
Antibody blood sampling
Reminder phone or field worker contact
*The first 5 participants will be vaccinated sequentially


Stage 2 in Africa
Children 24 to 59 months of age
Vaccination
Safety Blood sampling
Antibody blood sampling
Reminder phone or field worker contact
20 children*
Control or Dose B
20 children*
Control or Dose C
Screening
Visit 1
Visit 2
Visit 3
Visit 4
Visit 5
Visit 6
Day -21 to -1
Day 1
Day 8
Day 29
Day 85
Day 92
Day 113
Day 4 or Day 1 to Day 7
Day 88 or Day 85 to Day 91
*The first 5 participants for each dose group will be vaccinated sequentially

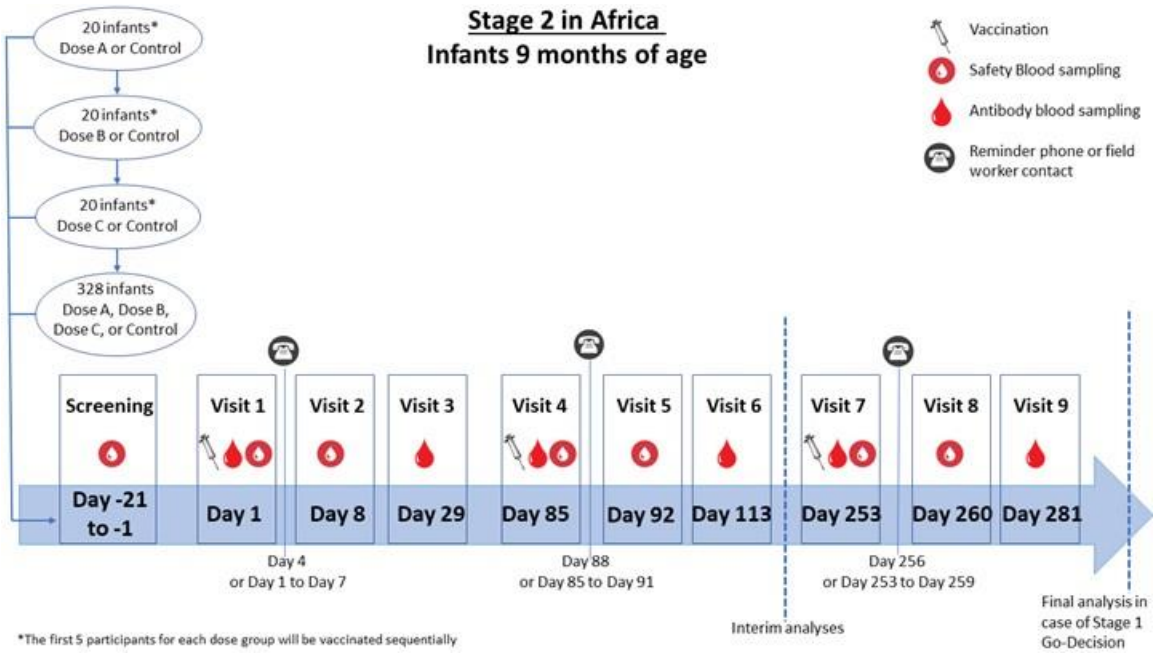

Stage 2 in Africa
Infants 9 months of age
Vaccination
Safety Blood sampling
Antibody blood sampling
Reminder phone or field worker contact
20 infants*
Dose A or Control
20 infants*
Dose B or Control
20 infants*
Dose C or Control
328 infants
Dose A, Dose B, Dose C, or Control
Screening
Visit 1
Visit 2
Visit 3
Visit 4
Visit 5
Visit 6
Visit 7
Visit 8
Visit 9
Day -21 to -1
Day 1
Day 8
Day 29
Day 85
Day 92
Day 113
Day 253
Day 260
Day 281
Day 4 or Day 1 to Day 7
Day 88 or Day 85 to Day 91
Day 256 or Day 253 to Day 259
Interim analyses
Final analysis in case of Stage 1 Go-Decision
*The first 5 participants for each dose group will be vaccinated sequentially

Table 2 Study groups, intervention and blinding in Stage 1

Study groups	Number of participants	Age (Min-Max)	Study intervention	Blinding
				Screening → Visit 7 (observer-blind)
ST1_Adults_Placebo_GR1	17	18 to 50 years (Adults)	altSonflex Placebo (Day 1 and Day 85)	●
ST1_Adults_Dose C_GR1	34	18 to 50 years (Adults)	Dose C altSonflex1-2-3 (Day 1 and Day 85)	●
ST1_Adults_Placebo_GR2	17	18 to 50 years (Adults)	altSonflex Placebo (Day 1 and Day 169)	●
ST1_Adults_Dose C_GR2	34	18 to 50 years (Adults)	Dose C altSonflex1-2-3 (Day 1 and Day 169)	●

Table 3 Study groups, intervention and blinding in Adults in Stage 2

Study groups	Number of participants	Age (Min-Max)	Study intervention	Blinding
				Screening → Visit 6 (observer-blind)
ST2_Adults_Control C	10	18 to 50 years (Adults)	MENVEO (Day 1), BOOSTRIX (Day 85)	●
ST2_Adults_Dose C	10	18 to 50 years (Adults)	Dose C altSonflex1-2-3 (Day 1 and Day 85)	●

Table 4 Study groups, intervention and blinding in Children in Stage 2

Study groups	Number of participants	Age (Min-Max)	Study intervention	Blinding
				Screening → Visit 6 (observer-blind)
ST2_Children_Control B	10	24 to 59 months (Children)	MENVEO (Day 1), TYPHIM VI (Day 85)	●
ST2_Children_Dose B	10	24 to 59 months (Children)	Dose B altSonflex1-2-3 (Day 1 and Day 85)	●
ST2_Children_Control C	10	24 to 59 months (Children)	MENVEO (Day 1), TYPHIM VI (Day 85)	●
ST2_Children_Dose C	10	24 to 59 months (Children)	Dose C altSonflex1-2-3 (Day 1 and Day 85)	●

Table 5 Study groups, intervention and blinding in Infants in Stage 2

Study groups	Number of participants	Age (Min-Max)	Study intervention	Blinding
				Screening → Visit 9 (observer-blind)
ST2_Infants_Control A_Safety	10	9 months (Infants)	MENVEO (Day 1 and Day 85), MR-VAC (Day 29 and Day 281), INFANRIX HEXA (Day 253)	●
ST2_Infants_Dose A_Safety	10	9 months (Infants)	Dose A altSonflex1-2-3 (Day 1, Day 85, and Day 253), MR-VAC (Day 29 and Day 281)	●
ST2_Infants_Control B_Safety	10	9 months (Infants)	MENVEO (Day 1 and Day 85), MR-VAC (Day 29 and Day 281), INFANRIX HEXA (Day 253)	●
ST2_Infants_Dose B_Safety	10	9 months (Infants)	Dose B altSonflex1-2-3 (Day 1, Day 85, and Day 253), MR-VAC (Day 29 and Day 281)	●
ST2_Infants_Control C_Safety	10	9 months (Infants)	MENVEO (Day 1 and Day 85), MR-VAC (Day 29 and Day 281), INFANRIX HEXA (Day 253)	●
ST2_Infants_Dose C_Safety	10	9 months (Infants)	Dose C altSonflex1-2-3 (Day 1, Day 85, and Day 253), MR-VAC (Day 29 and Day 281)	●
ST2_Infants_Control_Dose find	82	9 months (Infants)	MENVEO (Day 1 and Day 85), MR-VAC (Day 1 and Day 253), INFANRIX HEXA (Day 253)	●
ST2_Infants_Dose A_Dose find	82	9 months (Infants)	Dose A altSonflex1-2-3 (Day 1, Day 85, and Day 253), MR-VAC (Day 1 and Day 253)	●
ST2_Infants_Dose B_Dose find	82	9 months (Infants)	Dose B altSonflex1-2-3 (Day 1, Day 85, and Day 253), MR-VAC (Day 1 and Day 253)	●
ST2_Infants_Dose C_Dose find	82	9 months (Infants)	Dose C altSonflex1-2-3 (Day 1, Day 85, and Day 253), MR-VAC (Day 1 and Day 253)	●

4. ANALYSIS SETS

4.1. Definition

Table 6 Analysis Sets

Analysis set	Description
Enrolled	All participants who entered the study (who were randomised or received study intervention or underwent a post-screening study procedure). Note: participants who never passed screening (screening failures) and participants screened but who never enrolled into the study (met eligibility criteria but were not needed) are excluded from the Enrolled analysis set.
Exposed	All participants who received at least 1 dose of the study intervention. The allocation in a group is based on the administered intervention.
Full Analysis	All participants who received at least 1 dose of the study intervention and have post-vaccination immunogenicity data. The allocation in a group is done in function of the received intervention. The Full Analysis Set for immunogenicity will be defined by time point.
Per Protocol	All eligible participants who received all doses as per protocol, had immunogenicity results post-dose, complied with dosing/blood draw intervals, without intercurrent conditions that may interfere with immunogenicity and without prohibited concomitant medication/vaccination. The Per Protocol Set (PPS) for immunogenicity will be defined by time point.
Unsolicited Safety	All participants who received at least 1 dose of the study intervention (Exposed Set) that report unsolicited AEs/report not having unsolicited AEs. The allocation in a group is based on the administered intervention.
Solicited Safety	All participants who received at least 1 dose of the study intervention (Exposed Set) who have solicited safety data.

4.2. Criteria for eliminating data from Analysis Sets

Elimination codes are used to identify participants to be eliminated from analysis. Detail is provided below for each set.

4.2.1. Elimination from Enrolled Set

Code 700 (Screening failure), 800 (Fraudulent data) and code 900 (invalid informed consent) will be used for identifying participants eliminated from Enrolled Set.

4.2.2. Elimination from Exposed Set (ES)

Code 700 (Screening failure), Code 1030 (Study intervention not administered at all), 800 (Fraudulent data) and code 900 (invalid informed consent) will be used for identifying participants eliminated from ES.

4.2.3. Elimination from Full Analysis Set (FAS)

A participant will be excluded from FAS analysis under the following conditions:

- For codes 700, 800, 900 and 1030: participants will be eliminated for all visits.
- For codes 2100 and 2120 participants will be eliminated at the specific visit the condition is met.

Code	Condition under which the code is used	Visit (timepoints) where the code is applicable			Impacted Visit (timepoints)
		Adults in Europe	**Adults and Children in Africa**	**Infants in Africa**	**All participants**
700	Screening failure	All	All	All	All
800	Fraudulent data	All	All	All	All
900	Invalid informed consent or informed consent deviation	All	All	All	All
1030	Study vaccine not administered at all	All	All	All	All
2100	Serological results not available	Visit 1, 3, 4, 5, 7	Visit 1, 3, 4, 6	Visit 1, 3, 4, 6, 7, 9	Visit in which the condition is met
2120	Obvious incoherence, abnormal serology evolution or error in data (incoherence between CRF and results, wrong sample labelling)	Visit 1, 3, 4, 5, 7	Visit 1, 3, 4, 6	Visit 1, 3, 4, 6, 7, 9	Visit in which the condition is met

Code 2120 should be evaluated carefully after immunological results unblinding and it is out of scope for interim analyses.

4.2.4. Elimination from Per Protocol Set (PPS)

All eligible participants who received each dose as per protocol, had immunogenicity results post-dose, complied with dosing/blood draw intervals, without intercurrent conditions that may interfere with immunogenicity and without prohibited concomitant medication/vaccination are included in the PPS by time point.

4.2.4.1. Excluded participants

A participant will be excluded from analysis under the following conditions:

- For codes 700, 800, 900, 1030, 1050 and 1060: participants will be eliminated for all visits.
- For codes 1040, 1070, 1080, 1090, 2010, 2040, 2050, 2060, 2070 and 2080: participants will be eliminated from the specific visit in which the condition is met and all the subsequent visits.

- For codes 2090, 2100, 2120 and 2130: participants will be eliminated at the specific visit in which the condition is met.

Code	Condition under which the code is used	Visit (timepoints) where the code is applicable			Impacted Visit (timepoints)
		Adults in Europe	**Adults and Children in Africa**	**Infants in Africa**	**All participants**
700	Screening failure	All	All	All	All
800	Fraudulent data	All	All	All	All
900	Invalid informed consent or informed consent deviation	All	All	All	All
1030	Study vaccine not administered at all	All	All	All	All
1040	Administration of concomitant vaccine(s) forbidden in the protocol	All	All	All	Visit in which the condition is met and onwards
1045	Administration of allowed* per protocol concomitant vaccine(s) outside the recommended time intervals (at least 7 days before or 7 days after, 14 days in case of live vaccines)	All	All	All	Visit in which the condition is met or next visits relevant to this specific vaccination**
1050	Randomisation failure (randomised to the wrong group, subject randomized out of order)	All	All	All	All
1060	Randomisation code was broken	All	All	All	All
1070	Vaccination not according to protocol	Visit 1, 5	Visit 1, 4	Visit 1, 4, 7	Visit in which the condition is met and onwards
1080	Vaccine temperature deviation (no GMP use)	Visit 1, 5	Visit 1, 4	Visit 1, 4, 7	Visit in which the condition is met and onwards

Code	Condition under which the code is used	Visit (timepoints) where the code is applicable			Impacted Visit (timepoints)
		Adults in Europe	**Adults and Children in Africa**	**Infants in Africa**	**All participants**
1090	Expired vaccine administered	Visit 1, 5	Visit 1, 4	Visit 1, 4, 7	Visit in which the condition is met and onwards
2010	Eligibility criteria not met	Visit 1, 5	Visit 1, 4	Visit 1, 4, 7	Visit in which the condition is met and onwards
2040	Administration of any medication or medical device forbidden by the protocol	All	All	All	Visit in which the condition is met and onwards
2050	Not withdrawn after developing withdrawal criteria other than prohibited concomitant vaccination/medication: Underlying medical condition forbidden by the protocol	All	All	All	Visit in which the condition is met and onwards
2060	Not withdrawn after developing withdrawal criteria other than prohibited concomitant vaccination/medication: Infection related to the vaccine	All	All	All	Visit in which the condition is met and onwards
2070	Not withdrawn after developing withdrawal criteria other than prohibited concomitant vaccination/medication: Confirmed infection not related to the vaccine other than COVID-19	All	All	All	Visit in which the condition is met and onwards

Code	Condition under which the code is used	Visit (timepoints) where the code is applicable			Impacted Visit (timepoints)
		Adults in Europe	Adults and Children in Africa	Infants in Africa	All participants
2080	Participants did not comply with vaccination schedule	Visit 1, 5	Visit 1, 4	Visit 1, 4, 7	Visit in which the condition is met and onwards
2090	Participants did not comply with blood sample schedule for immunogenicity evaluation	Visit 1, 3, 4, 5, 7	Visit 1, 3, 4, 6	Visit 1, 3, 4, 6, 7, 9	Visit in which the condition is met
2100	Serological results not available post-vaccination for immunogenicity evaluation	Visit 1, 3, 4, 5, 7	Visit 1, 3, 4, 6	Visit 1, 3, 4, 6, 7, 9	Visit in which the condition is met
2120	Obvious incoherence, abnormality, or error in data (incoherence between CRF and results, wrong sample labelling)	Visit 1, 3, 4, 5, 7	Visit 1, 3, 4, 6	Visit 1, 3, 4, 6, 7, 9	Visit in which the condition is met
2130	Testing performed on samples not matching or consistent with participant ICF	Visit 1, 3, 4, 5, 7	Visit 1, 3, 4, 6	Visit 1, 3, 4, 6, 7, 9	Visit in which the condition is met

*Vaccines allowed per protocol Section 5.2.2 include emergency mass vaccination for an unforeseen public health threat, flu vaccines and COVID-19 vaccines in all participants, and EPI vaccines in children and infants.
**e.g. If an infant gets an allowed vaccine 3 days before visit 4 (2nd vaccination), he/she will be excluded from the PPS for visit 4 and 6. The flags to be used for exclusion during programming should be PPSV4FL and PPSV6FL. If an infant gets an allowed vaccine 3 days after visit 4 (2nd vaccination), he/she will be excluded from the PPS for visit 6. The flag to be used for exclusion during programming should be PPSV6FL

Code 2120 should be evaluated carefully after immunological results unblinding and it is out of scope for interim analyses.

4.2.5. Elimination from Safety Sets

4.2.5.1. Unsolicited Safety Set

A participant will be excluded from analysis under the following conditions:

- For codes 700, 800, 900 and 1030: participants will be eliminated for all visits.
- For codes 1150 participants will be eliminated at the specific visit the condition is met.

Code	**Condition under which the code is used**	**Visit (timepoints) where the code is assessed**			**Impacted analysis timepoint**
		Adults in Europe	**Adults and Children in Africa**	**Infants in Africa**	**All participants**
700	Screening failure	All	All	All	All
800	Fraudulent data	All	All	All	All
900	Invalid informed consent deviation or informed consent deviation	All	All	All	All
1030	Study vaccine not administered at all	All	All	All	All
1150	No post-vaccination unsolicited safety data	Visit 4, 7	Visit 3, 6	Visit 3, 6 9	Closest vaccination visit preceding assessment visit

4.2.5.2. Solicited Safety Set

A participant will be excluded from analysis under the following conditions:

- For codes 700, 800, 900 and 1030: participants will be eliminated for all visits.
- For code 1160 participants will be eliminated at the specific visit the condition is met.

Code	Condition under which the code is used	Visit (timepoints) where the code is assessed			Impacted analysis timepoint
		Adults in Europe	Adults and Children in Africa	Infants in Africa	All participants
700	Screening failure	All	All	All	All
800	Fraudulent data	All	All	All	All
900	Invalid informed consent deviation or informed consent deviation	All	All	All	All
1030	Study vaccine not administered at all	All	All	All	All
1160	No post-vaccination solicited safety data	Visit 2, 6	Visit 2, 5	Visit 2, 5, 8	Closest vaccination visit preceding assessment visit

5. STATISTICAL ANALYSES

Standard data derivation rules and statistical methods are described in Section 10.1 while the study specific data derivation rules and statistical methods are described in Section 9.

5.1. Analysis of demography and baseline characteristics

5.1.1. Analysis planned in the protocol

Demography and baseline characteristics are not described in the protocol.

5.1.2. Additional considerations

Demographic characteristics (age at first study intervention administration in months or years, sex, race and ethnicity) will be summarized by treatment group and overall using descriptive statistics:

- Frequency tables will be generated for categorical variable such as sex and race.
- Mean, median, standard deviation (SD) and range will be provided for continuous data such as age, height/length, weight, and body-mass index (as applicable).

The analysis population will be the Enrolled Set, Exposed Set and Per Protocol Set.

If, in any study group of infant dose-finding cohort and at any timepoint, the percentage of participants receiving study intervention with serological results excluded from the PPS for analysis of immunogenicity is 10% or more of the ES, the demographic analysis will be performed on the ES.

Withdrawal from study status will be summarized by group using descriptive statistics:

- The numbers of participants withdrawn from the study will be tabulated according to the reason for withdrawal. Table will be repeated specifying if there is any relationship of withdrawal with COVID-19 pandemic.
- The number of subjects enrolled into the study as well as the number of subjects excluded from each analysis set will be tabulated.

Vital signs will be summarized by vaccine group using descriptive statistics at all timepoint(s) the information is collected on Exposed Set.

Summary of medical history will be performed on Exposed Set by Medical Dictionary for Regulatory Activities (MedDRA) System Organ Class and Preferred Term.

Summary of participants taking a concomitant medication/vaccination within 28 days following each dose and overall will be performed on Exposed Set.

A summary of COVID-19 assessments throughout the study will be tabulated for each group.

Summary of important protocol deviations leading to elimination will be tabulated by vaccine group. An individual listing will also be provided.

For Stage 1, vaccinees will be assessed separately by clinical schedule (Dose C GR1, Dose C GR2) and pooled (Dose C GR1+ Dose C GR2). Placebo recipients will be always pooled. In Stage 2, participants receiving Control vaccination will be pooled within the same age safety cohort (dose finding cohort data will not be pooled with safety cohorts data and will be presented separately).

Tables will be generated separately for Adults in Europe, Adults in Africa, Children in Africa, Infants in Africa safety cohort and Infants in Africa dose-finding cohort.

5.2. Immunogenicity

5.2.1. Analysis planned in the protocol

The analysis on primary objective will be based on the PPS for analysis of immunogenicity. If, in any study group at timepoint of primary objective, the percentage of vaccinated participants with serological results excluded from the PPS for analysis of immunogenicity is 10% or more, a second analysis based on the ExposedSet will be performed to complement the per-protocol analysis.

Primary immunogenicity endpoint:

Within-groups assessment

For each study group (Dose A, Dose B, Dose C, and control vaccine) of the 9-month infant dose-finding cohort, the GMC with 95% CI of anti-serotype specific Shigella LPS* total IgG as measured by GVGH ELISA will be calculated at 28 days after third vaccination.

Between groups assessment

For each of the 4 serotypes in 9-month infant dose-finding cohort, the preferred dose at 28 days after third vaccination will be identified according to the scenarios reported in SAP Figure 2:

- Scenario 1 and 2: Dose A
- Scenario 3: t-test comparing Dose C with Dose A
- Scenario 4: t-test comparing Dose B with Dose A
- Scenario 5: hierarchical sequential with the 3 following tests:

1. t-test comparing Dose C with Dose A: if null hypothesis is rejected then
2. t-test comparing Dose B with Dose A: if null hypothesis is rejected then
3. t-test comparing Dose B with Dose C.

In addition to scenarios above and in Figure 2, in case of a dose level discontinuation

- if the lower dose among the 2 remaining doses is more immunogenic at 28 days after third vaccination, then the lower dose will be selected as the preferred dose without performing any statistical test
- if the higher dose among the 2 remaining doses is more immunogenic at 28 days after third vaccination, then a t- test comparing the higher with lower dose will be performed to select the preferred dose

Unadjusted Ratios of GMCs with 95% CI between groups for all pairwise comparisons will be calculated.

Secondary immunogenicity endpoint:

Within-groups assessment

For each study group in participants in Europe, for each sampling timepoint, and for each antigen, the following statistics will be computed:

- Unadjusted GMCs with 95% CI of anti-serotype specific Shigella LPS/OAg* total IgG as measured by GVGH ELISA
- Within-participant geometric mean ratios (GMRs) will be computed for GMCs with 95% CI at each time point after vaccination versus baseline (Day 1)

- Number and percentage of participants with 4-fold increase compared to pre-vaccination, 1:800, and 1:1600 equivalent titres with exact 95% CI as measured by GVGH ELISA

For each study group in participants in Africa, for each sampling timepoint, and for each antigen, the following statistics will be computed:

- Unadjusted GMCs with 95% CI of anti-serotype specific Shigella LPS/OAg* total IgG as measured by GVGH ELISA
- Within-participant GMRs will be computed for GMCs with 95% CI at each time point after vaccination versus baseline (Day 1)
- Number and percentage of participants with 4-fold increase compared to pre-vaccination, 1:800, and 1:1600 equivalent titres with exact 95% CI as measured by GVGH ELISA

For each study group within 9-month dose-finding infant cohort, for the sampling timepoint before the first vaccination and 28 days after the second MR-VAC vaccination, the following statistics will be computed for the anti-measles and anti-rubella antigens, as measured by ELISA:

- Unadjusted GMCs with 95% CI of anti-measles and anti-rubella antibodies
- Percentage of participants with measles seroresponse of ≥150 mIU/mL and ≥200 mIU/mL, with exact 95% CI
- Percentage of participants with rubella seroresponse of ≥4 mIU/mL and ≥10 mIU/mL, with exact 95% CI.

For all the above, within-participant GMRs will be computed for GMT or GMC with 95% CI at each time point after vaccination versus baseline (Day 1).

The distribution of antibody concentrations for each antigen will be displayed using reverse cumulative distribution curves.

**S. sonnei, S. flexneri 1b, S. flexneri 2a, and S. flexneri 3a* serotypes will be tested.

5.2.2. Additional considerations

For primary immunogenicity endpoints:

Tables, figures and listings will be generated for Infants in Africa dose-finding cohort.

Within-groups assessment

The antibody concentration will be logarithmically transformed (base10) to fulfil the normal distribution assumption. GMCs will be calculated at 28 days after third vaccination, with their associated two-sided 95% CIs, by exponentiating (base 10) the means of log-transformed titres and their 95% CIs. Additionally, within-participant geometric mean ratios (GMRs) will be computed for geometric mean titres at 28 days

after third vaccination versus baseline (Day 1) and versus pre-vaccination. The GMRs and 95% CIs will be constructed by exponentiating (base 10) the means of within-participant differences between log-transformed titres after third vaccination and log-transformed titres at baseline/pre-vaccination, and their corresponding 95% CIs. GMRs will be calculated only for participants with valid baseline/pre-vaccination immunogenicity results.

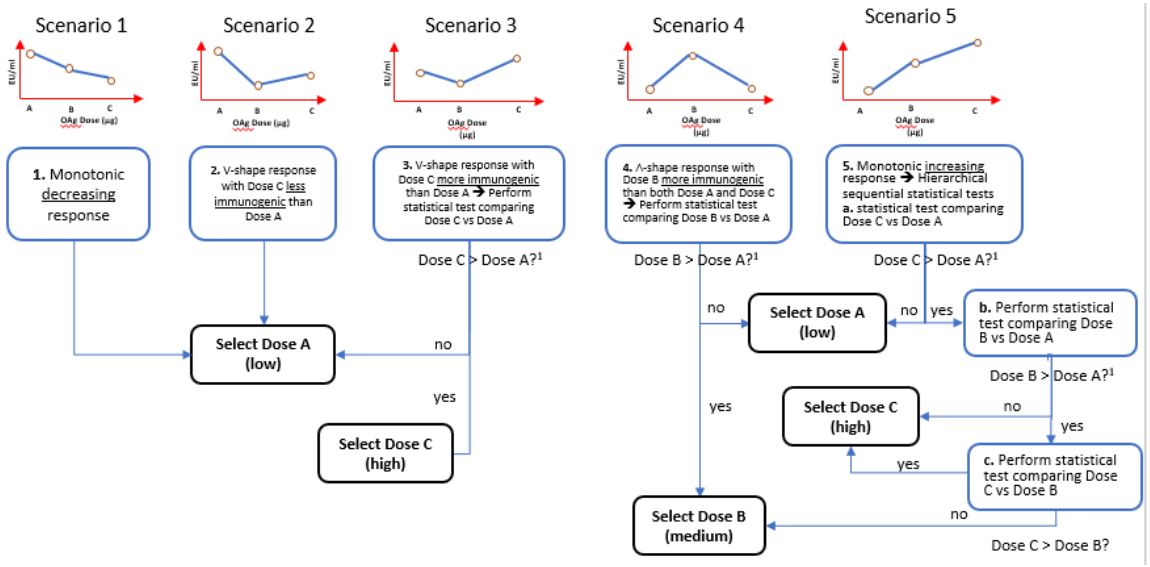
The distribution of serotype specific Shigella anti-LPS/OAg serum IgG for study groups at 28 days after third vaccination will be displayed graphically using reverse cumulative distribution curves for all serotypes (*S. sonnei*, *S. flexneri* 1b, *S. flexneri* 2a, and *S. flexneri* 3a).

ELISA IgG concentration distribution against serotype specific Shigella anti-LPS/OAg serum IgG at 28 days after third vaccination for study groups for each serotype will be displayed graphically using boxplots.

Between groups assessment

The immunogenic dose-response shape expressed as GMCs measured by GVGH ELISA will be displayed through figures for each serotype. These figures will match with one of the 5 foreseen scenarios in Figure 2. The identification of the preferred dose for each component will be possible using the above selection strategy, by advising this figure and consulting the table showing difference between groups (Dose C vs Dose A, Dose B vs Dose A, Dose B vs Dose C) as ratio of GMCs along with their CIs and p-values obtained by two-sided two-independent sample t-test. Measurements will not be adjusted for multiple comparisons.

Figure 2 Dose-response scenarios and statistical tests for identifying the preferred dose of the altSonflex1-2-3 vaccine



For secondary immunogenicity endpoints:

- The antibody concentrations will be logarithmically transformed (base10) to fulfil the normal distribution assumption. GMCs will be calculated, with their associated two-sided 95% CIs, by exponentiating (base 10) the means of log-transformed concentrations/titres and their 95% CIs.
- Within-participant geometric mean ratios (GMRs) will be computed for geometric mean concentrations at each sampling timepoint versus baseline (Day 1) and versus pre-vaccination. The GMRs and 95% CIs will be constructed by exponentiating (base 10) the means of within-participant differences between log-transformed titres after each sampling timepoint and log-transformed titres at baseline/ pre-vaccination, and their corresponding 95% CIs. GMRs will be calculated only for participants with valid baseline/ pre-vaccination immunogenicity results.
- Percentage of participants with 4-fold increase compared to baseline (Day 1) and pre-vaccination and their exact 95% CIs will be tabulated.
- Percentage of participants with ELISA level equal to or above 1:800, 1:1600 titre/equivalent titre against *S. sonnei* LPS and their exact 95% CIs will be tabulated.
- The distribution of serotype specific Shigella anti-LPS/OAg serum IgG, anti-measles and anti-rubella IgG concentration for study groups at sampling timepoints will be displayed graphically using reverse cumulative distribution curves.
- ELISA IgG concentration distribution against serotype specific Shigella anti-LPS/OAg serum IgG at sampling timepoints for study groups for each serotype will be displayed graphically using boxplots.
- Anti-measles and anti-rubella IgG distribution of concentrations at sampling timepoints for study groups will be displayed graphically using boxplots.

The analysis population for secondary immunogenicity endpoints will be the Per Protocol Set.

Cohen's equivalent threshold (1:800 titres) and double of Cohen's equivalent threshold (1:1600 titres) [Cohen, 1988] will be used for statistical analysis. Individual sera ran in GVGH ELISA, reference ELISA (WRAIR ELISA) and Cohen's ELISA, to establish commutability of results as obtained by the three methodologies, and especially to establish the antibody level corresponding to the median titre reported in convalescent sera [Cohen, 1988] and the double of this (1:800 and 1:1600 in Cohen's ELISA, respectively). Analysis of results obtained in the three labs performed by NIBSC estimated equivalent of 800 and 1600 titres in Tel Aviv Assay (TAU) as per table below.

TAU titre	Estimated GVGH EU (95% CI)	Estimated WRAIR titre (95% CI)
800	161 (124 – 211)	1053 (703 – 1577)
1600	396 (315 – 497)	2550 (1811 – 3590)

It has been decided for analyses purposes to consider as equivalent for each threshold the Lower Limit of the 95% CI of the point estimate. (i.e. 124 EU/mL and 315 EU/mL for GVGH ELISA and 703 titres and 1811 titres for reference ELISA, for 800 and 1600 TAU titres respectively). The decision to consider the LL of 95% CI and not the point estimate was made in order to maximise the probability of excluding participants above threshold at baseline.

Analysis will be performed as described above for all participants and may be repeated for participants with GVGH ELISA level < 1:800 equivalent titres and <1:1600 equivalent titres at baseline, for *S. sonnei* serotype.

All the immunogenicity analyses will be stratified by the following subgroups:

- Participants with antibodies above Lower Limit of Quantification (LLOQ) at baseline
- Participants with antibodies below LLOQ at baseline

The cut-off values for each serotype were defined by the local laboratory.

VISMEDERI laboratory provided the LLOQ cut off values as described in table below.

Serotype	**Method**	**Cut Off value**	**Unit**
S sonnei	GVGH ELISA	12.8	EU/mL
S flexneri 1b	GVGH ELISA	6	EU/mL
S flexneri 2a	GVGH ELISA	3	EU/mL
S flexneri 3a	GVGH ELISA	2.9	EU/mL

Values below LLOQ will be set to half the LLOQ value for analysis purpose.

If baseline value is below LLOQ then 4-fold is defined as 4 times the LLOQ cut off value. If baseline value is above or equal to LLOQ then 4-fold is defined as 4 times the baseline value.

For Stage 1, vaccinees will be assessed separately by clinical schedule (Dose C GR1, Dose C GR2) and pooled (Dose C GR1+ Dose C GR2). Placebo recipients will be always pooled. In Stage 2, participants receiving Control vaccination will be pooled within the same age safety cohort (dose finding cohort data will not be pooled with safety cohorts data and will be presented separately).

Tables/figures/listings will be generated separately for Adults in Europe, Adults in Africa, Children in Africa, Infants in Africa safety cohort and Infants in Africa dose-finding cohort.

5.2.2.1. Sensitivity analyses

A sensitivity analysis will be performed for the between groups assessment of primary immunogenicity endpoint to investigate impact of baseline titres on the choice of preferred dose. For each serotype, GMCs, ratio of GMCs along with their p-values and 95% CIs will be obtained from an Analysis of Covariance (ANCOVA) model with baseline titres and treatment (i.e. Dose A, Dose B, Dose C) as covariates.

A second sensitivity analysis will be performed for the between groups assessment of primary immunogenicity endpoint to investigate impact on enlarged sample size on choice of preferred dose pooling dose-finding cohort and safety cohort of infants receiving vaccine Dose A, Dose B and Dose C. For each serotype, GMCs, ratio of GMCs along with their p-values and 95% CIs will be obtained from an Analysis of Covariance (ANCOVA) model with baseline titres, cohort and treatment (i.e. Dose A, Dose B, Dose C) as covariates.

5.2.2.2. Quantitative Decision Making

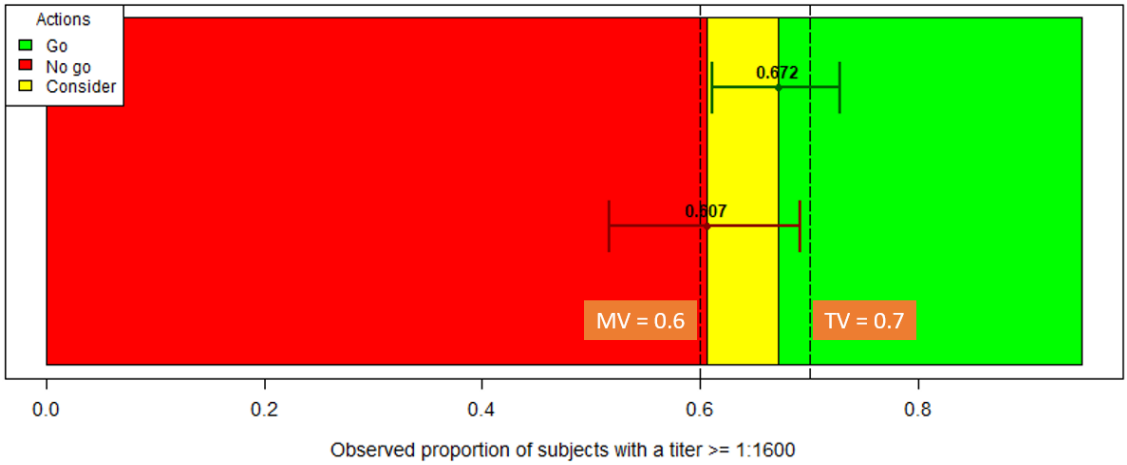
QDM will be assessed for participants in the PPS.

The critical values of 60.7% and 67.2% were calculated as follows:

- The critical value associated with Minimum Value (MV) is the smallest point estimate such that the lower limit of one-sided 80% exact Confidence Interval for proportion of participants achieving an equivalent 1:1600 ELISA level against *S. sonnei* titres is above or equal to the MV.
- The critical value associated with Target Value (TV) is the highest point estimate such that the upper limit of one-sided 90% exact Confidence Interval for proportion of participants achieving an equivalent 1:1600 ELISA level against *S. sonnei* titres is below or equal to the TV.

As critical values depend on the number of evaluable participants, they will be recalculated at the time of the analysis.

Figure 3 QDM Critical values and decisions



MV=minimum value; TV=target value

5.3. Analysis of safety and reactogenicity

5.3.1. Analysis planned in the protocol

Primary safety endpoints:

- The percentage of participants with at least 1 administration-site solicited event or unsolicited AE, with at least 1 systemic solicited event or unsolicited AE and with any AE during the 7-day or 28-day follow-up period will be tabulated by group after each vaccine dose and overall.
- The percentage of doses associated with at least 1 administration-site solicited event or unsolicited AE, with at least 1 systemic event and unsolicited AE, and with any AE will be tabulated for the overall vaccination course. The same calculations will be performed for symptoms rated as Grade 3.
- The percentage of participants reporting each individual solicited administration-site and systemic event (any Grade and Grade 3) during the 7-day solicited follow-up period will be tabulated. The percentage of doses associated with each individual solicited administration-site and systemic event will be tabulated for the overall vaccination course.
- Occurrence of fever will be reported as number and percentages of participants reporting temperature ≥38°C in 0.5°C cumulative increments during the 7-day follow-up period. This will be tabulated for each group after each dose.The percentage of participants with at least one reported unsolicited AE during the 28-day follow-up period after each vaccination as classified by the Medical Dictionary for Regulatory Activities (MedDRA) preferred term will be tabulated. The same tabulation will be performed for Grade 3 unsolicited AEs and for unsolicited AEs according to relationship to vaccination.
- Percentage of participants reporting SAEs and AEs/SAEs leading to withdrawal from the study during the participant period participation will be tabulated. The detailed listings will also be produced.
- For each group and for haematological, renal, and hepatic panel test results at 7 days after each vaccination:
- The percentage of participants having haematological, renal, and hepatic results below or above the laboratory normal ranges will be tabulated by time point.
- The maximum Grade (refer to SAP Section 9.1) from Day 1 up to the end of the participant's participation will be tabulated.

The verbatim reports of unsolicited symptoms will be reviewed by a physician and the signs and symptoms will be coded according to the MedDRA Dictionary for AE Terminology.

5.3.2. Additional considerations

Safety analysis will be based on the Exposed Set, Solicited Safety Set, and Unsolicited Safety Set.

In Stage 1, vaccinees receiving Dose C will be assessed separately for each clinical schedule (i.e. Dose C GR1, Dose C GR2) and pooled regardless of the clinical schedule (i.e. Dose C GR1+ GR2), for all timepoints. Placebo recipients will be always pooled. In Stage 2, participants receiving Control vaccination will be pooled within the same age safety cohort (dose finding cohort data will not be pooled with safety cohorts data and will be presented separately).

Tables/listings will be generated separately for Adults in Europe, Adults in Africa, Children in Africa, Infants in Africa safety cohort and Infants in Africa dose-finding cohort.

5.3.2.1. Analysis of solicited events

Solicited events analysis will be based on the Solicited Safety Set.

Compliance in completing solicited events information will be provided by day after each vaccination for each group.

For solicited events, missing or unevaluable measurements will not be replaced. Therefore, the analysis of the solicited symptoms will include only subjects/doses with documented safety data. Details of missing or unevaluable measurements are provided in SAP Section 10.1.2.3

The only solicited systemic event to be analysed for this study is fever.

The solicited administration site events to be analysed are pain, redness and swelling.

Details of solicited events grading are provided in SAP Section 9.1 .

Number and percentage of participants with at least one administration site, systematic solicited event (fever) and any event within 7 days following each dose and overall will be summarized along with the exact 95% CIs for each group. Analysis will be repeated for grade 3 solicited AEs.

Number and percentage of participants with fever/administration site solicited events by maximum intensity within 7 days following each dose and overall will be summarized along with the exact 95% CIs for each group. All grading categories will be presented i.e Any grade, Grade 1, Grade 2, Grade 3, At least Grade 1, At least Grade 2. Fever will be additionally reported as number and percentages of participants reporting temperature ≥38°C in 0.5°C cumulative increments. Same analysis will be performed for the percentage of doses associated with each individual solicited administration-site and systemic event. Analysis will be repeated for fever related to vaccination.

5.3.2.2. Analysis of unsolicited AEs and SAEs

The analysis of unsolicited AEs and SAEs will be performed on the Unsolicited Safety Set/Exposed Set as applicable.

All the unsolicited adverse events occurring during 28 days after vaccination will be judged either as related or not related to vaccination by the investigator. Same assessment will be done for SAEs during entire study period.

The original verbatim terms used by investigators to identify adverse events in the e-CRFs will be mapped to preferred terms using the MedDRA dictionary. The unsolicited adverse events will then be grouped by MedDRA preferred terms into frequency tables according to system organ class. Adverse events judged by the investigator as at least possibly related to study vaccine will be summarized by vaccine group, according to System Organ Class (SOC) and Preferred Term (PT) within SOC. 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.

Number and percentage of participants with at least one unsolicited AE (by Primary System Organ Class and Preferred Term) and any AE within 28 days following each dose and overall will be summarized along with exact 95% CIs for each group. Analysis will be repeated for related/grade 3 unsolicited AEs.

Number and percentage of occurrences and participants with at least one serious related/medically attended/ leading to withdrawal unsolicited AE (by Primary System Organ Class and Preferred Term) and any AE during the entire study period will be summarized with exact 95% CIs for each group.

Summary of participants by unsolicited AE category (related/serious/medically attended/fatal) within 28 days following any dose will be summarized along with exact 95% CIs for each group.

To assess the impact of the COVID-19 pandemic on the safety results, the overall incidence of COVID-19 reported as AEs and SAEs and COVID-19 AEs leading to study withdrawal, will be summarized with exact 95% CIs for each group.

5.3.2.3. Analysis of solicited events and unsolicited AEs

The combined analysis of solicited and unsolicited AEs will be performed on the Exposed Set as requested for public disclosure.

Solicited events and unsolicited AEs will be coded by MedDRA.

Summary of occurrences and participants with at least one serious adverse event during their entire study period (including any SAEs, fatal SAEs, related SAEs and fatal related SAEs) will be summarized.

Summary of occurrences and participants with at least one solicited or unsolicited adverse event, excluding SAE, with onset within 28 days of any vaccination will be summarized. This is the only case where solicited and unsolicited AEs will be analysed pooled.

5.3.2.4. Other safety analyses

All collected concomitant medications and vaccinations will be listed. In addition, concomitant medication will be summarized by any antipyretic, any antibiotic or any medication. In case of pregnancies during the study, follow-up data and pregnancy outcomes will be described in detail.

For all subjects in each group and each **haematology and biochemistry** parameter:

- The number and percentage of subjects having haematology, renal and hepatic panel test results below, within or above the local laboratory normal ranges will be tabulated as baseline value versus values 7 days after each vaccination and overall. Parameters in scope of evaluation are described in Protocol Table 27.
- The number and percentage of subjects having haematology, renal and hepatic panel test results will be tabulated as baseline value versus values 7 days after each vaccination and overall, by adequate grading scale for each age group as described in SAP Section 9.1

Baseline will be considered the pre-vaccination value before each vaccination.

In case of repeated tests performed within a visit, the intensity of the first test will be taken in consideration for the tabulation. In listings all values will be shown.

The analysis will be performed on the Exposed Set.

In addition, SAEs related to study participation or concurrent GSK medication/vaccine reported from inform consent signed until entire study duration will be also listed.

5.4. Tertiary endpoints

5.4.1. Analysis planned in the protocol

Tertiary immunogenicity endpoints in scope of this SAP:

From the subset defined by 25% randomly chosen participants from the Stage 1 adults 18 to 50 years of age, “Stage 1 Adults Subset”, the following statistics will be computed:

- Unadjusted GMTs with 95% CI of anti-serotype specific* serum bactericidal antibody, before, 15 days after first vaccination, and 28 days after each vaccination
- Anti-serotype serum bactericidal antibody GMTs against a panel of vaccine heterologous Shigella serotypes, as measured by SBA.

For participants in Africa in the safety cohorts, for each sampling timepoint, and for each antigen the following statistics will be computed, as measured by reference ELISA:

- Unadjusted GMTs with 95% CI of anti-serotype specific Shigella LPS* total IgG
- Within-participant GMRs will be computed for GMTs with 95% CI at each time point after vaccination versus baseline
- Number and percentage of participants with 1:800 and 1:1600 equivalent titres with exact 95% CI

From the subset defined by randomly chosen participants from the Stage 2 adults 18 to 50 years of age (“Stage 2 Adults Subset”), children 24 to 59 months of age (“Stage 2 Children Subset”) and infant 9 month of age safety cohort ("Stage 2 Infant Safety Cohort Subset"), the following statistics will be computed:

- Unadjusted GMTs with 95% CI of anti-serotype specific* serum bactericidal antibody, before the first vaccination and 28 days after each vaccination

For all participants in the 9-month infant dose-finding cohort, the following statistics will be computed

- Unadjusted GMTs with 95% CI of anti-serotype specific* serum bactericidal antibody, before and 28 days after each vaccination

For a selected subset of participants in the 9-month infant dose-finding cohort, “Stage 2 Infants Dose-Finding Cohort Subset”, the following statistics will be computed

- Anti-serotype serum bactericidal antibody GMTs against a panel of vaccine heterologous Shigella serotypes, as measured by SBA

For all the above, within-participant GMRs will be computed for GMT with 95% CI at each time point after vaccination versus baseline. Anti-*Shigella* conserved protein IgG ELISA assays will be performed on the aforementioned subsets of 25% randomly chosen Stage 1 adult participants and on the infant dose-finding cohort to be defined. Results will be reported by group and timepoint using descriptive summary statistics.

5.4.2. Additional considerations

For the subset defined by 25% randomly chosen participants from Stage 1 adults 18 to 50 years of age the following statistics will be computed:

- Number and percentage of participants with 4-fold increase compared to pre-vaccination with exact 95% CI of anti-serotype specific serum bactericidal antibody, 15 days after first vaccination, and 28 days after each vaccination
- Unadjusted GMTs with 95% CI of anti-serotype serum bactericidal antibody against a panel of vaccine heterologous Shigella serotypes, at pre-vaccination and 28 days each vaccination

- Number and percentage of participants with 4-fold increase compared to pre-vaccination with exact 95% CI of anti-serotype serum bactericidal antibody against a panel of vaccine heterologous Shigella serotypes, 28 days after each vaccination

For participants in Africa in the safety cohorts, for each antigen the following statistics will be computed, as measured by reference ELISA:

- Number and percentage of participants with 4-fold increase compared to baseline with exact 95% CI at 28 days after each vaccination

For a subset of participants in Africa safety cohorts the following statistics will be computed:

- Number and percentage of participants with 4-fold increase compared to baseline (Day 1) with exact 95% CI of anti-serotype specific serum bactericidal antibody, 28 days after each vaccination

For infants from the 9-month safety cohort in Africa, the following statistics will be computed:

- Unadjusted GMTs with 95% CI of anti-Shigella-conserved protein IgG, as measured by anti-protein ELISA, at pre-vaccination and 28 days each vaccination
- Number and percentage of participants with 4-fold increase compared to pre-vaccination with exact 95% CI of anti-Shigella-conserved protein IgG, as measured by anti-protein ELISA, 28 days after each vaccination

For the 9-month infant dose-finding cohort, the following statistics will be computed:

- Number and percentage of participants with 4-fold increase compared to pre-vaccination with exact 95% CI of anti-serotype specific serum bactericidal antibody, 28 days after each vaccination.
- Unadjusted GMTs with 95% CI of anti-Shigella-conserved protein IgG, as measured by anti-protein ELISA, at pre-vaccination and 28 days each vaccination
- Number and percentage of participants with 4-fold increase compared to pre-vaccination with exact 95% CI of anti-Shigella-conserved protein IgG, as measured by anti-protein ELISA, 28 days after each vaccination

For a subset of 9-month infant dose-finding cohort, the following statistics will be computed:

- Unadjusted GMTs with 95% CI of anti-serotype serum bactericidal antibody against a panel of vaccine heterologous Shigella serotypes, at pre-selected timepoints
- Number and percentage of participants with 4-fold increase compared to pre-vaccination with exact 95% CI of anti-serotype serum bactericidal antibody against a panel of vaccine heterologous Shigella serotypes, at pre-selected timepoints

The analysis of above mentioned tertiary endpoints will be based on PPS or a subset of PPS for analysis of immunogenicity

The antibody titres will be logarithmically transformed (base10) to fulfil the normal distribution assumption. GMTs will be calculated, with their associated two-sided 95% CIs, by exponentiating (base 10) the means of log-transformed concentrations/titres and their 95% CIs.

Within-participant geometric mean ratios (GMRs) will be computed for geometric mean titres at each sampling timepoint versus baseline (Day 1) and versus pre-vaccination. The GMRs and 95% CIs will be constructed by exponentiating (base 10) the means of within-participant differences between log-transformed titres after each sampling timepoint and log-transformed titres at baseline/pre-vaccination, and the corresponding 95% CIs. GMR will be calculated only for participants with valid baseline/pre-vaccination valid immunogenicity results.

Additionally, analyses will be stratified by the following subgroups:

- Participants with antibodies equal or above LLOQ at baseline
- Participants with antibodies below LLOQ at baseline

GVGH laboratory provided the LLOQ cut off values as described in table below.

Serotype	**Method**	**Cut Off value**	**Unit**
S sonnei	L-SBA	33	IC50
S flexneri 1b (Stage 1)	L-SBA	21.8	IC50
S flexneri 1b (Stage 2)	L-SBA	42	IC50
S flexneri 2a	L-SBA	13.4	IC50
S flexneri 3a (Stage 1)	L-SBA	82.6	IC50
S flexneri 3a (Stage 2)	L-SBA	21	IC50
S flexneri 4a	L-SBA	8	IC50
S flexneri 5b	L-SBA	26	IC50
S flexneri 6a	L-SBA	18	IC50
S flexneri X	L-SBA	68	IC50
S flexneri Y	L-SBA	14	IC50

The cut-off values of anti-protein ELISA assay for each serotype will be defined by the GVGH laboratory as soon as characterization will be finished.

WRAIR laboratory provided the LLOQ cut off values as described in table below.

Serotype	**Method**	**Cut Off value**	**Unit**
S sonnei	Reference ELISA	100	Titre
S flexneri 1b	Reference ELISA	100	Titre
S flexneri 2a	Reference ELISA	100	Titre
S flexneri 3a	Reference ELISA	100	Titre

Values below LLOQ will be set to half the LLOQ value for analysis purpose.

If baseline value is below LLOQ then 4-fold is defined as 4 times the LLOQ cut off value. If baseline value is above or equal to LLOQ then 4-fold is defined as 4 times the baseline value.

Additionally, for Stage 1 Adults Subset, Stage 2 Adults Subset, Stage 2 Children Subset, Stage 2 Infant Safety Cohort Subset, and Stage 2 infants dose-finding cohort the correlation between anti-serotype specific bactericidal antibodies titres (as measured by SBA) and anti-serotype specific Shigella LPS/OAg total IgG antibody concentration (as measured by GVGH ELISA) will be assessed by Pearson correlation coefficient, calculated on the log10 transformed values, at each timepoint and overall. Graphical representation of the same will be performed via scatter plots.

For Stage 1, vaccinees will be assessed separately by clinical schedule (Dose C GR1, Dose C GR2) and pooled (Dose C GR1+ Dose C GR2). Placebo recipients will be always pooled. In Stage 2, participants receiving Control vaccination will be pooled within the same age safety cohort (dose finding cohort data will not be pooled with safety cohorts data and will be presented separately).

Tables/listings will be generated separately for Adults in Europe, Adults in Africa, Children in Africa, Infants in Africa safety cohort and Infants in Africa dose-finding cohort.

Tables of anti-Shigella-conserved protein IgG will be generated separately and pooled for Infants in Africa safety cohort and Infants in Africa dose-finding cohort. Code similar to Section 10.1.6 may be used.

Tertiary endpoints may be included in interim/final analysis according to data availability from GVGH and WRAIR laboratories.

6. ANALYSIS INTERPRETATION

Statistical hypothesis testing will be applied to the following primary hypothesis:

- To identify the preferred dose of each component of the altSonflex1-2-3 (low, medium, or high) for infants 9 months of age in Africa (Stage 2).

The statistical tests will be chosen according to the dose-response relationship that will be observed for each of the 4 components of the vaccine. See Section 5.2.2 of the SAP for more details.

No statistical hypothesis testing will be performed for all other objectives.

All other analyses are descriptive, and the use of these descriptive analyses will be limited to supportive analyses of confirmatory analyses or hypotheses generation.

7. INTERIM ANALYSES

7.1. Sequence of analyses

Description	**Disclosure Purpose (CTRS=public posting, SR=study report, internal)**
Interim 1 Stage 1 (ANALYSIS_E1_02)	Internal, sharing with Bill and Melinda Gates Foundation (BMGF)
Interim 2 Stage 1 (ANALYSIS_E1_03)	Internal, sharing with BMGF/ Public disclosure (CTRS)*
Interim 3 Stage 2 (ANALYSIS_E1_04)	Internal, sharing with BMGF
Interim 4 Stage 2 (ANALYSIS_E1_05)	Internal, sharing with BMGF
Final Analysis (ANALYSIS_E1_01)	Public disclosure, Study report

*Public disclosure (CTRS) in case of No-Go decision after Stage 1 interim analysis at Day 29

All analyses before unblinding will be conducted on cumulative data as clean as possible.

The sequence of interim and final analyses will be the following:

a. Stage 1 interim analysis at Day 29 (28 days after first vaccination) on immunogenicity in all Stage 1 participants. This analysis will support a QDM approach, which will be part of a GO/NO-GO decision for the study to proceed to Stage 2; to be carried out by the IDAC. No individual listings or data with the participants identifying information will be disseminated.

b. Stage 1 interim analysis at Day 197 on immunogenicity and safety data on all Stage 1 participants in order to obtain early immunogenicity and safety data from Stage 1; to be carried out by the IDAC. No individual listings or data with the participants identifying information will be disseminated.

c. Stage 2 interim analysis at Day 113 on immunogenicity data from all infant safety cohorts (28 days after their second vaccination) and on cumulative available immunogenicity data from all Stage 2 safety cohorts, in order to obtain early immunogenicity data analysis and facilitate planning for subsequent clinical development, to be carried out by the IDAC. No individual listings or data with the participants identifying information will be disseminated.

d. Stage 2 interim analysis at Day 113 on immunogenicity data from infant dose-finding cohort (28 days after their second vaccination) and on cumulative available immunogenicity data from all Stage 2 cohorts, to facilitate planning for subsequent clinical development, to be carried out by the IDAC. No individual listings or data with the participants identifying information will be disseminated.

e. Final analysis including Stage 1 final analysis at Day 197 and Stage 2 final analysis at Day 281 on all immunogenicity and safety data once Stage 2 of the trial is concluded; to be carried out by the GVGH Project Statistician and GSK Statistical Analysts.

In case of a No-Go decision after the first (a) or second (b) interim analyses, a final analysis will be performed at Day 197 on immunogenicity and safety data of all Stage 1 participants, to be carried out by GVGH Project Statistician and GSK Statistical Analysts.

The Clinical Study Report (CSR) will contain at least the final analyses of all primary and secondary endpoints (in case of a Go decision). Only Stage 1 endpoints will be part of the CSR in case of a No-Go decision (interim analyses “a” and “b”).

Data for tertiary endpoints which are available at the time of planned analyses will be included in the interim analyses. Data for tertiary endpoints that become available at later timepoints will be documented in annex(es) to the final CSR or in an integrated final CSR.

For interim analysis d, complete data summarized in previous interim analyses are not in scope for cumulative reporting.

7.2. Statistical considerations for interim analyses

No adjustment of Type I error will be performed since no statistical hypothesis testing will be conducted prior to unblinding.

8. CHANGES TO THE PROTOCOL DEFINED STATISTICAL ANALYSIS

Second line analysis for primary objective will be done on ES instead of the FAS so that the participants will be analysed as treated. Reason for this change is that for the purpose of dose-selection analysis is of most value to analyse data according to the actual vaccination received.

9. NON-STANDARD DATA DERIVATION RULES AND STATISTICAL METHODS

The following sections describe additional derivation rules and statistical methods which are not presented in Section 10.1.

9.1. Data derivation

The intensity of the following solicited events will be assessed as described:

Infant/ Child (<6 years)		
Event	**Intensity grade**	**Parameter**
Pain at administration site	0	None
	1	Mild: Minor reaction to touch
	2	Moderate: Cries/protests on touch
	3	Severe: Cries when limb is moved/spontaneously painful
Redness at administration site		Greatest surface diameter in mm
Swelling at administration site		Greatest surface diameter in mm
Temperature*		Temperature in °C (with 1 decimal)

*Fever is defined as temperature ≥38.0°C. The preferred location for measuring temperature will be the axilla for all participants.

Adults		
Event	**Intensity grade**	**Parameter**
Pain at administration site	0	None
	1	Mild: Any pain neither interfering with nor preventing normal everyday activities
	2	Moderate: Painful when limb is moved and interferes with everyday activities
	3	Severe: Significant pain at rest. Prevents normal everyday activities
Redness at administration site		Greatest surface diameter in mm
Swelling at administration site		Greatest surface diameter in mm
Temperature*		Temperature in °C (with 1 decimal)

*Fever is defined as temperature ≥38.0°C. The preferred location for measuring temperature will be the axilla for all participants.

In infants and children, the maximum intensity of local injection-site erythema/swelling will be scored as follows:

Redness/Swelling (Infants/Children)			
Grade 0 **Absent**	**Grade 1** **Mild**	**Grade 2** **Moderate**	**Grade 3** **Severe**
<1 mm	≥1 – ≤25 mm	>25 – ≤50 mm	>50 mm

In adults, the maximum intensity of local injection-site erythema/swelling will be scored as follows:

Redness/Swelling (Adults)			
Grade 0 **Absent**	**Grade 1** **Mild**	**Grade 2** **Moderate**	**Grade 3** **Severe**
<20 mm	≥20 – ≤50 mm	>50 – ≤100 mm	>100 mm

In all participants, the maximum intensity of fever will be scored as follows:

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

The investigator will assess the maximum intensity that occurred over the duration of the event for all unsolicited AEs (including SAEs) recorded during the study. The assessment will be based on the investigator's clinical judgement.

The intensity should be assigned to one of the following categories:

1 (mild)	=	An AE which is easily tolerated by the participant, causing minimal discomfort and not interfering with everyday activities.
2 (moderate)	=	An AE which is sufficiently discomforting to interfere with normal everyday activities.
3 (severe)	=	An AE which prevents normal, everyday activities (In a young child, such an AE would, for example, prevent attendance at a day-care centre and would cause the parent(s)/LAR(s) to seek medical advice. In adults, such an AE would, for example, prevent attendance at work and would necessitate the administration of corrective therapy.)

An AE that is assessed as Grade 3 (severe) should not be confused with an SAE. Grade 3 is a category used for rating the intensity of an event; and both AEs and SAEs can be assessed as Grade 3. An event is defined as 'serious' when it meets 1 of the predefined outcomes as described in Table 7.

Grading scales for haematology and biochemistry safety laboratory assessments are reported below.

Table 7 Definition of Serious Adverse Event (SAE)

An SAE is any untoward medical occurrence that meets 1 of the followings:
a. Results in death.
b. Is life-threatening Note: The term 'life-threatening' in the definition of 'serious' refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, had it been more severe.
c. Requires hospitalisation or prolongation of existing hospitalisation Note: In general, hospitalisation signifies that the participant has been admitted at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or in an out-patient setting. Complications that occur during hospitalisation are also considered AEs. The event will also be considered serious if a complication prolongs hospitalisation or fulfils any other serious criteria. When in doubt as to whether 'hospitalisation' occurred or was necessary, the AE should be considered serious.
d. Results in disability/incapacity Note: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhoea, influenza like illness, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.
e. Is a congenital anomaly/birth defect in the offspring of a study participant.
f. Abnormal pregnancy outcomes (e.g. spontaneous abortion, foetal death, stillbirth, congenital anomalies, ectopic pregnancy).
g. Other situations Medical or scientific judgement must be exercised in deciding whether reporting is appropriate in other situations. Important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the participant or require medical or surgical intervention to prevent one of the other outcomes listed in the above definition should be considered serious. Examples of such events are invasive or malignant cancers; intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias; and convulsions that do not result in hospitalisation.

Grading scales for haematology and biochemistry safety laboratory assessments

Stage 1 – Adults (Europe)

Laboratory test	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially life-threatening (Grade 4)
Creatinine – mg/dL (male)	>1.17 – 1.7	>1.7 – 2.0	>2.0 – 2.5	>2.5 or requires dialysis
Creatinine – mg/dL (female)	>0.96 – 1.7	>1.7 – 2.0	>2.0 – 2.5	>2.5 or requires dialysis
Liver Function Tests –ALT, AST increase by factor	≥1.1 – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 – 10 x ULN	>10 x ULN
Haemoglobin (female) - g/dL	11.0 – <11.8	9.5 – <11.0	8.0 – <9.5	< 8.0
Haemoglobin (female/male) change from baseline value - g/dL	Any decrease – 1.5	>1.5 – 2.0	>2.0 – 5.0	> 5.0
Haemoglobin (male) - g/dL	12.5 – <12.9	10.5 – <12.5	8.5 – <10.5	< 8.5
WBC Increase - cell/mm^3	>9300 – 15 000	>15 000 – 20 000	>20 000 – 25 000	> 25 000
WBC Decrease - cell/mm^3	2500 – <3650	1500 – <2500	1000 – <1500	<1000
Platelets Decreased - cell/mm^3 (male)	125 000 – <149 000	100 000 – <125 000	25 000 – <100 000	<25 000
Platelets Decreased – cell/mm^3 (female)	125 000 – <171 000	100 000 – <125 000	25 000 – <100 000	<25 000
Neutrophils Decrease - cell/mm^3	1500 – <1573	1000 – <1500	500 – <1000	<500
Lymphocytes decrease cell/mm^3	750 - <1133	500 - <750	250 - <500	<250
Eosinophils Cell/mm^3	>273 - 1500	>1500 - 5000	>5000	Hypereosinophilic

ALT=alanine aminotransferase; AST aspartate aminotransferase; ULN=upper limit of normal; WBC=white blood cell
The grading scale has been adapted from the Food and Drug Administration (FDA) Guidance for Industry- Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials [U.S. Department of Health and Human Services, 2007] and adjusted considering local normal ranges provided by the site. Parameters not included in the FDA grading scales will not be graded; their assessment will be based on laboratory normal ranges and medical judgement.

Stage 2 – Adults (Africa)

Laboratory test	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially life-threatening (Grade 4)
Creatinine	≥1.1 – 1.3 x ULN	>1.3 – 1.8 x ULN OR Increase to 1.3 to <1.5 x participant's baseline	>1.8 – <3.5 x ULN OR Increase to 1.5 to <2.0 x participant's baseline	≥3.5 x ULN OR Increase of ≥2.0 x participant's baseline
Liver Function Tests –ALT, AST	≥1.25 – <2.5 x ULN	2.5 – <5.0 x ULN	5.0 – <10.0 x ULN	≥10.0 x ULN
Haemoglobin (female) - g/dL	9.5	8.5 – < 9.5	6.5 – < 8.5	< 6.5
Haemoglobin (male) - g/dL	10.0 – <13.3	9.0 – < 10.0	7.0 – < 9.0	< 7.0
WBC Decrease - cell/mm^3 (male)	2000 – <2700	1500 – <2000	1000 – <1500	<1000
WBC Decrease - cell/mm^3 (female)	2000 – <3000	1500 – <2000	1000 – <1500	< 1000
Platelets Decreased - cell/mm^3 (male)	100 000 – <115 000	50 000 – < 100 000	25 000 – <50 000	<25 000
Platelets Decreased - cell/mm^3 (female)	100 000 – <124 000	50 000 – <100 000	25 000 – <50 000	<25 000
Neutrophils Decrease - cell/mm^3 (male)	800 – <870	600 – <800	400 – <600	<400
Neutrophils Decrease - cell/mm^3 (female)	800 – <962	600 – <800	400 – <600	<400
Lymphocytes decrease cell/mm^3 (male)	600 – <1098	500 – <600	350 – <500	<350
Lymphocytes decrease cell/mm^3 (female)	600 – <1264	500 – <600	350 – <500	<350

ALT=alanine aminotransferase; AST aspartate aminotransferase; ULN=upper limit of normal; WBC=white blood cell
The grading scale has been adapted from the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events [U.S. Department of Health and Human Services, 2017] and adjusted considering local normal ranges provided by the site. Parameters not included in the DAIDS grading scales will not be graded; their assessment will be based on laboratory normal ranges and medical judgement.

Stage 2 – Children (Africa)

Laboratory test	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially life-threatening (Grade 4)
Creatinine	≥1.1 – 1.3 x ULN	>1.3 – 1.8 x ULN OR Increase to 1.3 to < 1.5 x participant's baseline	>1.8 – <3.5 x ULN OR Increase to 1.5 to < 2.0 x participant's baseline	≥3.5 x ULN OR Increase of ≥2.0 x participant's baseline
Liver Function Tests –ALT, AST	≥1.25 – <2.5 x ULN	2.5 – <5.0 x ULN	5.0 – <10.0 x ULN	≥10.0 x ULN
Haemoglobin (female & male) - g/dL	9.5 – <10.2	8.5 – < 9.5	6.5 – <8.5	<6.5
WBC Decrease - cell/mm^3 (male)	2000 – <5140	1500 – <2000	1000 – <1500	<1000
WBC Decrease - cell/mm^3 (female)	2000 – <4860	1500 – <2000	1000 – <1500	<1000
Platelets Decreased - cell/mm^3 (male)	100 000 – <202 000	50 000 – <100 000	25 000 – <50 000	<25 000
Platelets Decreased - cell/mm^3 (female)	100 000 – <189 000	50 000 – <100 000	25 000 – < 50 000	<25 000
Neutrophils Decrease - cell/mm^3 (male)	800 – <1540	600 – <800	400 – <600	< 400
Neutrophils Decrease - cell/mm^3 (female)	800 – <1600	600 – <800	400 – <600	<400

ALT=alanine aminotransferase; AST aspartate aminotransferase; ULN=upper limit of normal; WBC=white blood cell
The grading scale has been adapted from the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events [U.S. Department of Health and Human Services, 2017] and adjusted considering local normal ranges provided by the site. Parameters not included in the DAIDS grading scales will not be graded; their assessment will be based on laboratory normal ranges and medical judgement.

Stage 2 – Infants (Africa)

Laboratory test	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially life-threatening (Grade 4)
Creatinine µmol/L	≥40.7 – 48.1	>48.1 – 66.6	>66.6 – <129.5	≥129.5
Liver Function Tests –ALT, AST	≥1.25 – < 2.5 x ULN	2.5 –< 5.0 x ULN	5.0 – < 10.0 x ULN	≥ 10.0 x ULN
Haemoglobin (Female) - g/dL	9.5 – <10.2	8.5 – < 9.5	6.5 – < 8.5	<6.5
Haemoglobin (Male) - g/dL	9.5 – <10.1	8.5 – < 9.5	6.5 – < 8.5	<6.5
WBC Decrease - cell/mm^3 (male)	2000 – < 5980	1500 – <2000	1000 – <1500	<1000
WBC Decrease - cell/mm^3 (female)	2000 – <6480	1500 – <2000	1000 – <1500	<1000
Platelets Decreased - cell/mm^3 (male)	100 000 – <206 000	50 000 – <100 000	25 000 – <50 000	<25 000
Platelets Decreased - cell/mm^3 (female)	100 000 – <214 000	50 000 – <100 000	25 000 – <50 000	<25 000
Neutrophils Decrease - cell/mm^3 (male)	800 – <1190	600 – <800	400 – <600	<400
Neutrophils Decrease - cell/mm^3 (female)	800 – <1270	600 – <800	400 – <600	<400

ALT=alanine aminotransferase; AST aspartate aminotransferase; ULN=upper limit of normal; WBC=white blood cell
The grading scale has been adapted from the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events [U.S. Department of Health and Human Services, 2017] and adjusted considering local normal ranges provided by the site. Parameters not included in the DAIDS grading scales will not be graded; their assessment will be based on laboratory normal ranges and medical judgement.

9.2. Statistical method

9.2.1. Adjusted GMT or GMC ratios

Adjusted GMTs, their ratios and CIs will be obtained using an ANCOVA model on the logarithm transformed titres. The ANCOVA model will include the vaccine group as the fixed effect and the log-transformed titres values at baseline as covariate. GMT ratios and their 95% CIs will be derived by exponential (base 10) transformation of the corresponding group contrast in the model.

When between-group GMT or GMT ratios are computed and adjusted for two-level categorical co-variables (i.e. cohort dummy variable identifying which step infants are coming from), these co-variables should be included as dummy variables in the SAS procedure. It should also be clear whether the model is limited to the data from some groups.

10. ANNEXES

10.1. Business rules for standard data derivations and statistical methods

This section contains standard rules for data display and derivation for clinical and epidemiological studies. These rules will be applied along with those detailed in Section 9 (additional study-specific rules).

10.1.1. Attributing events to vaccine doses

The dose relative to an event is the most recent study dose given to a participant prior to the start of a given event. For example, if the start date of an adverse event is between Dose 1 and Dose 2, the relative dose will be Dose 1.

If an event starts on the same day as a study dose, the relative dose will be derived from the additional information provided in the case report form (CRF) using the contents of the flag indicating if the event occurred before or after study dose. If 'after study dose' is selected, the relative dose for the event will be the one administered on the start day of the event. If 'before study dose' is selected, the relative dose for the event will be the dose prior to this one.

10.1.2. Handling of missing data

10.1.2.1. Dates

When partially completed dates (i.e. dates missing a day and/or month) are used in calculations, the following standard rules will be applied:

- A missing day will be replaced by 15
- A missing day and month will be replaced by June 30th.

The following exceptions apply:

- Adverse event start dates with missing day:
  - If the event starts in the same month as at least one of the study doses, the contents of AE.AESTRTPT (the flag indicating if the event occurred before or after study dose) will be used to complete the date. If 'after study dose' or blank is selected, the imputed start date will match the first (or only) study dose given during that month. If 'before study dose' is selected, the imputed date will be one day before the first (or only) study dose given during that month.
- Adverse event start dates with missing day and month:
  - If the event starts in the same year as at least one of the study doses, the contents of AE.AESTRTPT (the flag indicating if the event occurred before or after study dose) will be used to complete the date. If 'after study dose' is selected, the imputed start date will match the first (or only) study dose given during that year. If 'before study dose' is selected, the imputed date will be one day before the first (or only) study dose given during that year.

- Adverse event end date with missing day.
  - The imputed end date should be set to the earliest of the (date of next treatment-1, last day of the month (taking into account leap years)).
- Adverse event end date with missing day and month.
  - The imputed end date should be set to the earliest of the (date of next treatment-1, last visit date for that subject, 31DECYYYY).
- Adverse event end date completely missing
  - For events that are still ongoing at the time of analysis an imputed end date based on the data lock point or the last visit date for that subject should be used for the calculation of duration.

All other cases of incomplete AE or concomitant medication/vaccination start date will follow the standard rules above.

If, after imputation, the end date of an event occurs before the start date, both will be given the same date. The chosen date should be the start date, unless the end date is recorded with more precision whereby the end date should be used.

10.1.2.2. Laboratory data

Missing laboratory results (including immunological data) will not be replaced.

Haematology/chemistry laboratory data requiring grading as indicated in Section 9.1 may have more decimals than expected or may require conversion to the unit associated to the grade leading to more decimals than expected (eg. Calcium results may be 7.91).

In order to determine the grading, the following rule will be used

1. In case a conversion is needed, the original results will be used for the conversion without a previous rounding.

10.1.2.3. Daily recording of solicited events

10.1.2.3.1. Studies with paper diaries

For studies using paper diaries which have questions in the CRF indicating the presence or absence of solicited events, the following rules are applicable:

- Denominators for the summary of administration site (or systemic) solicited events will be calculated using the number of participants who respond “Yes” or “No” to the question concerning the occurrence of administration site (or systemic) events.
- When a specific solicited event is marked as having not occurred following a specific study dose (i.e. SDTM CE.CEOCCUR=N for the specified post-dose period for the event in question), all daily measurements will be imputed as Grade 0.

- When a specific solicited event is marked as having occurred following a specific study dose (i.e. SDTM CE.CEOCCUR=Y for the specified post-dose period for the event in question), any missing daily recordings will be given imputed values to allow them to contribute to the 'Any' rows but not to specific grade rows of the solicited event summary tables.
- When the occurrence of a specific solicited event is not present (i.e. SDTM CE.CEOCCUR is neither Y nor N for the specified post-dose period for the event in question) but the group of solicited events (administration site) is marked as having occurred (i.e. SDTM CE.CEOCCUR=Y), all missing daily recordings will be given imputed values to allow them to contribute to the 'Any' rows but not to specific grade rows of the solicited event summary tables.
- When the occurrence of fever is not present (i.e. SDTM CE.CEOCCUR is neither Y nor N for the specified post-dose period for the event in question), missing daily recordings will be given imputed values to allow them to contribute to the 'Any' rows and "Unknown" grade category.

The following table shows how participants contribute to each category for a specific solicited event over the Day X to Day Y post-dose period:

Solicited event category	Participants included in the calculation of the numerator
Any	All participants with at least one occurrence of the adverse event at grade 1, grade 2, or grade 3 between Day X and Day Y or with the adverse event marked as present and at least one missing daily recording between Day X and Day Y
At least grade 1	All participants with at least one occurrence of the adverse event at grade 1, grade 2, or grade 3 between Day X and Day Y
At least grade 2	All participants with at least one occurrence of the adverse event at grade 2 or grade 3 between Day X and Day Y
At least grade 3	All participants with at least one occurrence of the adverse event at grade 3 between Day X and Day Y

10.1.2.4. Unsolicited adverse events

Unsolicited adverse events summaries are including serious adverse events unless specified otherwise.

Missing severity, relationship with study vaccine, and outcome of unsolicited adverse events will not be replaced and will appear as 'UNKNOWN' when displayed in a statistical output.

10.1.3. Data derivation

10.1.3.1. Age at first dose in days

When age at first dose is to be displayed in days, it will be calculated as:

Age = date of first dose minus date of birth

10.1.3.2. Age at first dose in months

When age at first dose is to be displayed in months, it will be calculated as the difference in days of Date of first dose and date of birth (DOB), divided by 30.4375.

10.1.3.3. Age at first dose in years

When age at first dose is to be displayed in years, it will be calculated as the difference in days of Date of first dose and date of birth (DOB), divided by 365.25.

10.1.3.4. Weight

Weight will be presented in kilograms. Weights reported in pounds will be converted as follows:

Weight in kilograms = Weight in pounds / 2.2

10.1.3.5. Height

Height will be presented in centimeters. Heights reported in feet and inches will be converted as follows:

Height in centimeters = Height in inches x 2.54

10.1.3.6. Body mass index (BMI)

BMI will be calculated as follows:

BMI = (Weight in kilograms) / (Height in meters)^2

10.1.3.7. Temperature

Temperatures will be presented in degrees Celsius (°C). Temperatures reported in degrees Fahrenheit (°F) will be converted as follows:

Temperature (Celsius) = ((Temperature (Fahrenheit) - 32) x 5)/9

10.1.3.8. Numerical serology results

Numerical serology results will be derived from the content of IS.ISORRES in the SDTM dataset. For assays with a specific cut-off, the following derivation rules apply:

IS.ISORRES	Derived value
"NEG", "-", or "(-)"	cut-off/2
"POS", "+", or "(+)"	cut-off
"< value" and value is <= assay cut-off	cut-off/2
"< value" and value is > assay cut-off	value
"> value" and value is < assay cut-off	cut-off/2
"> value" and value is >= assay cut-off	value
"value" and value is < cut-off	cut-off/2
"value" and value is >= cut-off	value
All other cases	missing

10.1.3.9. Geometric mean titres (GMTs) and concentrations (GMCs)

Geometric Mean Titre (GMT) or Concentration (GMC) calculations are performed by taking the inverse logarithm of the mean of the log titre or concentration transformations. Non quantifiable antibody titres or concentrations will be converted as described in Section 10.1.3.8 for the purpose of GMT/GMC calculation. Cut-off values are defined by the laboratory before the analysis.

10.1.3.10. Onset day

The onset day for an event (e.g. AE, concomitant medication/vaccination) is the number of days between the last study dose and the start date of the event. This is 1 for an event occurring on the same day as a study dose (and reported as starting after study dose).

10.1.3.11. Duration of events

The duration of an event with a start and end date will be the difference between the end and start date plus one day, i.e. an event that starts on 03MAR2018 and ends on 12MAR2018 has a duration of 10 days.

10.1.3.12. Counting rules for combining solicited and unsolicited adverse events

For output combining solicited and unsolicited adverse events, all serious unsolicited adverse events will be considered systemic events since the administration site flag is not included in the expedited adverse event CRF pages. Unsolicited adverse events with missing administration site flag will also be considered systemic.

Multiple events with the same preferred term which start on the same day are counted as only one occurrence.

10.1.3.13. Counting rules for occurrences of solicited events

When the occurrences of solicited events are summarized, each event recorded as having occurred during a specific period will be counted as only one occurrence regardless of the number of days on which it occurs. Also, in the case of co-administered study interventions, an administration site event recorded for a participant following multiple study interventions will be counted as only one occurrence.

10.1.4. Display of decimals

10.1.4.1. Percentages

Percentages and their corresponding confidence limits will be displayed with one decimal except for 100% in which case no decimal will be displayed.

10.1.4.2. Differences in percentages

Differences in percentages and their corresponding confidence limits will be displayed with two decimals.

10.1.4.3. Demographic/baseline characteristics statistics

The mean, median, and standard deviation for continuous baseline characteristics (height, weight, body mass index (BMI), pre-dose body temperature) will be presented with one decimal.

The minimum and maximum values and quartile values (if required) will be presented with the same number of decimals as the observed values.

The maxima and minima of transformed height/weight variables will be displayed without decimals, with the exception of infant studies where one decimal will be displayed for the transformed weight.

The maximum and minimum of transformed body temperatures will be displayed with one decimal.

10.1.4.4. Serological summary statistics

The number of decimals used when displaying geometric mean titres (GMT) or concentrations (GMC) and their confidence limits is assay specific based on the magnitude of the assay result post-dose and the clinically relevant assay threshold. The same number of decimals will be used for a given assay regardless of the timepoint presented.

Lowest clinically relevant threshold	Example	Number of decimals to display
<0.3	Diphtheria, tetanus, anti-PRP	3
>=0.3 and <4	*Streptococcus pneumoniae*, Meningococal bactericide	2
>=4 and <1000	Measles, rubella, varicella, polio	1
>=1000	CMI	0

GMT/GMC fold increase from pre-dose follows the same principle. Namely when the lowest clinically relevant threshold is 2 fold, 2 decimals are displayed while when the lowest clinically relevant threshold is 4 fold, 1 decimal is displayed.

GMT or GMC group ratios and their confidence limits will be displayed with 2 decimals regardless of the actual values.

10.1.5. Statistical methodology

The exact confidence intervals around within-group proportions are derived using the method of Clopper and Pearson [Clopper, 1934].

10.1.6. SAS code

For sensitivity analysis adjusted for baseline values following code will be used:

```
proc sort data=immuno;
by visit;
run;
proc mixed data= immuno;
class treatment visit;
by visit;
model aval=treatment baseline/s alpha=0.05;
lsmeans treatment/ diff cl;
ods output LSMeans=GMT diffs=diff;
run;

data GMT;
set GMT;
GMT=10**estimate;
```

```
LL_95=10**Lower;
UL_95=10**Upper;
run;
```

Exponentiate to obtain ratio GM between vaccine and placebo/control

```
data diffdose;
set diffdose;
Ratio_GMT=10**estimate;
LL_95=10**lower;
UL_95=10**Upper;
run;
```

For sensitivity analysis adjusted for baseline values and cohort in all infants (safety and dose-finding cohort) code similar to the below will be used:

```
proc sort data=immuno;
by visit; run;
proc mixed data= immuno;
class treatment visit cohort;
by visit;
model aval=treatment baseline cohort / s alpha=0.05;
lsmeans treatment/ diff cl;
ods output LSMeans=GMT diffs=diff;
run;

data GMT;
set GMT;
GMT=10**estimate;
LL_95=10**Lower;
UL_95=10**Upper;
run;
```

Exponentiate to obtain ratio GM between vaccine and placebo/control

```
data diff;
set diff;
Ratio_GMT=10**estimate;
LL_95=10**lower;
UL_95=10**Upper;
run;
```

Code to calculate 95% CIs for immunogenicity and safety binary endpoints:

```
%let a=95;
if count = 0 then lcl = 0;
else lcl = (BETAINV(( 100-&a)/200, count,   N-count+1))*100;
if count = N then ucl = 100;
```

```
else ucl = (BETAINV(1-(100-&a)/200, count+1, N-count ))*100;
run;
```

Code to calculate 95% CIs for GMT/GMC:

```
%let alpha=5;
ods select basicintervals;
proc univariate data=isx cibasic(alpha=&alpha);
by arm armcd ordstrn istest visitnum;
var logres; *logres is 10log of “aval”;
ods output basicintervals=basicintervals;
run;
```

11. REFERENCES

Clopper CJ, Pearson E. The Use of Confidence or Fiducial Limits Illustrated in the case of the Binomial. *Biometrika.* 1934;26:404-13.

Cohen D, Green MS, Block C, et al. Serum antibodies to lipopolysaccharide and natural immunity to shigellosis in an Israeli military population. J Infect Dis. 1988;157:1068-71

Conti V, Rossi O, Clarkson KA, Mancini F, Nakakana UN, Sarakinou E, Callegaro A, Ferruzzi P, Acquaviva A, Arora AK, Marchetti E, Necchi F, Frenck RW Jr, Martin LB, Kaminski RW, Podda A, Micoli F. Putative correlates of protection against shigellosis assessing immunomarkers across responses to S. sonnei investigational vaccine. NPJ Vaccines. 2024 Mar 8;9(1):56. doi: 10.1038/s41541-024-00822-2. PMID: 38459072; PMCID: PMC10923941.

Launay O, Lewis DJM, Anemona A, et al. Safety profile and immunologic responses of a novel vaccine against *Shigella sonnei* administered intramuscularly, intradermally and intranasally: results from two parallel randomized Phase 1 clinical studies in healthy adult volunteers in Europe. EBioMedicine. 2017;22:164–72.

U.S. Department of Health and Human Services Food and Drug Administration (FDA) Center for Biologics Evaluation and Research. Guidance for Industry- Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials; September 2007. (https://www.fda.gov/regulatory-information/search-fda-guidance-documents/toxicity-grading-scale-healthy-adult-and-adolescent-volunteers-enrolled-preventive-vaccine-clinical). *Accessed 15 December 2020.*

U.S. Department of Health and Human Services. Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Pediatric Adverse Events. Corrected Version 2.1; July 2017. (https://rsc.niaid.nih.gov/clinical-research-sites/grading-severity-adult-pediatric-adverse-events-corrected-version-two-one). *Accessed 15 December 2020*.