Statistical Analysis Plan Amendment 3

Study ID: 208109

Official Title of Study: A Phase I, Single-center, Randomized, Observer-blind, Placebo-controlled Study to Evaluate Safety, Reactogenicity and Immunogenicity of GSK's Clostridium difficile Investigational Vaccine Based on the F2 Antigen With or Without AS01B Adjuvant, When Administered Intramuscularly According to a 0, 1-month Schedule to Healthy Adults Aged Between 18-45 Years and Between 50-70 Years, Followed by an Additional Dose Administered in a Partial blind Manner Within an Interval of Approximately 15 Months After Dose 2, in a Subcohort of Subjects aged 50-70 Years

NCT number: NCT04026009

Date of Document: 01 Oct 2021

STATISTICAL ANALYSIS PLAN

208109 (CDIFF-004)

A PHASE I, SINGLE-CENTER, RANDOMIZED, OBSERVER-BLIND, PLACEBO-CONTROLLED STUDY TO EVALUATE SAFETY, REACTOGENICITY AND IMMUNOGENICITY OF GSK'S *CLOSTRIDIUM DIFFICILE* INVESTIGATIONAL VACCINE BASED ON THE F2 ANTIGEN WITH OR WITHOUT $AS01_B$ ADJUVANT, WHEN ADMINISTERED INTRAMUSCULARLY ACCORDING TO A 0, 1-MONTH SCHEDULE TO HEALTHY ADULTS AGED BETWEEN 18-45 YEARS AND BETWEEN 50-70 YEARS, FOLLOWED BY AN ADDITIONAL DOSE ADMINISTERED IN A PARTIAL BLIND MANNER WITHIN AN INTERVAL OF APPROXIMATELY 15 MONTHS AFTER DOSE 2, IN A SUBCOHORT OF SUBJECTS AGED 50-70 YEARS

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VERSION NUMBER AND DATE: V3.0, 27SEP2021

STATISTICAL ANALYSIS PLAN SIGNATURE PAGE - AUTHORS

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STATISTICAL ANALYSIS PLAN SIGNATURE PAGE - APPROVAL

Upon review of this document, the undersigned approves this version of the Statistical Analysis Plan, authorizing that the content is acceptable for the reporting of this study.

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MODIFICATION HISTORY

Listed below are modifications made to the SAP after the signed approval(s).

Unique Identifier for this Version	Date of the Document Version	Author	Significant Changes from Previous Authorized Version
1.0	01AUG2019	PPD	Not Applicable – First Version
2.0	12JAN2021	PPD	COVID-19 impact related text added within various sections CCI [redacted]
3.0	27SEP2021	PPD	Added information about an additional third dose to be given to subjects on active treatment due to protocol amendment 03 dated 18MAY2021 Added information about second interim analyses Added information about analysis sets related to the Dose 3

TABLE OF CONTENTS

1. INTRODUCTION

This document describes the rules and conventions to be used in the presentation and analysis of safety and immunogenicity data at first (interim), second (interim) and final analyses for Protocol 208109 (CDIFF-004). It describes the data to be summarized and analyzed, including specifics of the statistical analyses to be performed. This statistical analysis plan (SAP) is based on protocol amendment 03 dated 18 May 2021. The iSRC/SRT analyses will be described in a separate document.

2. STUDY OBJECTIVES

2.1. PRIMARY OBJECTIVE

To evaluate the safety and reactogenicity profile of the *C. difficile* F2 fusion protein when administered with or without $AS01_B$ adjuvant as 2 intra-muscular (IM) doses according to a 0, 1-month schedule, in healthy adults aged between 18-45 years and between 50-70 years followed by an additional dose administered approximately 15 months after Dose 2 in a subcohort of subjects aged 50-70 years, during the entire study period

2.2. SECONDARY OBJECTIVES

To evaluate the humoral immunogenicity induced by 2 IM doses of the *C. difficile* F2 fusion protein when administered with or without $AS01_B$ adjuvant according to a 0, 1-month schedule, in healthy adults aged between 18-45 years and between 50-70 years followed by an additional dose administered approximately 15 months after Dose 2 in a subcohort of subjects aged 50-70 years, up to study end

3. STUDY DESIGN

3.1. GENERAL DESCRIPTION

This is a Phase I, first-in-human, single-center, randomized, observer-blind, placebo-controlled, study with 2 to 3 treatment groups per step in a 4-step staggered design in healthy subjects to generate safety, reactogenicity, and immunogenicity data for the development of a candidate *C. difficile* vaccine that would protect against primary cases of CDI and CDI recurrence.

A 4-step staggered design will be used to ensure maximum safety of the participating subjects:

Step 1: Vaccination of approximately 20 subjects aged 18-45 years (approximately 10 subjects in the

CDIFF antigen [Ag] group and approximately 10 subjects in the placebo group). An internal safety review committee (iSRC) will review all accumulating safety data after Dose 1 and then again after Dose 2. Vaccination in Step 2 will proceed in the absence of a safety concern detected by the iSRC on all accumulating safety data at the end of Step 1.

Step 2: Vaccination of approximately 20 subjects aged 50-70 years (approximately 10 subjects in the CDIFF Ag group and approximately 10 subjects in the placebo group). An iSRC will review all accumulating safety data after Dose 1 and then again after Dose 2. Vaccination in Step 3 will proceed in the absence of a safety concern detected by the iSRC on all accumulating safety data at the end of Step 2.

Step 3: Vaccination of approximately 30 subjects aged 50-70 years (approximately 10 subjects in the CDIFF Ag group, approximately 10 subjects in the CDIFF Ag + an adjuvant system containing 3-O-desacyl-4'-monophosphoryl lipid A [MPL], QS-21 and liposome [50 µg MPL and 50 µg QS-21] [$AS01_B$] group, and approximately 10 subjects in the placebo group). An iSRC will review all accumulating safety data after Dose 1 and then again after Dose 2. Vaccination in Step 4 will proceed in the absence of a safety concern detected by the iSRC on all accumulating safety data at the end of Step 3.

Step 4: Vaccination of approximately 70 subjects aged 50-70 years (approximately 25 subjects in the CDIFF Ag group, approximately 35 subjects in the CDIFF Ag + $AS01_B$ group, and approximately 10 subjects in the placebo group). An iSRC will review all accumulating safety data 3 weeks after the start of vaccination in Step 4 and then about every 3 weeks until all subjects have received Dose 1. In addition, the iSRC will review all safety data after Dose 2. A subcohort of subjects who have successfully completed all visits up to Visit 7, have received two doses on an active arm (ie, the subjects who received placebo will be excluded) do not meet any contraindication for subsequent vaccination, and still meet the eligibility criteria will receive a third dose after the iSRC has reviewed all the accumulating safety data at the end of Step 4 Dose 2. If there are any safety concerns observed during SRT reviews post Dose 3, an ad hoc iSRC meeting will take place to review the unblinded safety data.

During all 4 steps, all subjects should be closely observed for at least 60 minutes after vaccination. For Steps 1, 2, and 3 only, all subjects should be vaccinated sequentially and at least 60 minutes apart to allow for monitoring of any acute events (eg, hypersensitivity reaction). During vaccination days of Steps 1, 2, and 3 (ie, Day 1 and Day 31), vaccination will be limited to a maximum of 10 subjects per day. A total of 120 subjects aged 50 – 70 years (30 receiving placebo, 45 receiving CDIFF Ag and 45 receiving CDIFF Ag + $AS01_B$) and 20 subjects aged 18-45 years (10 receiving placebo and 10 receiving CDIFF Ag) will be enrolled in this study. The duration of the study for each subject receiving 2 doses will be approximately 1 year (Visit 7) and each subject receiving 3 doses will be approximately 2 years (Visit 11).

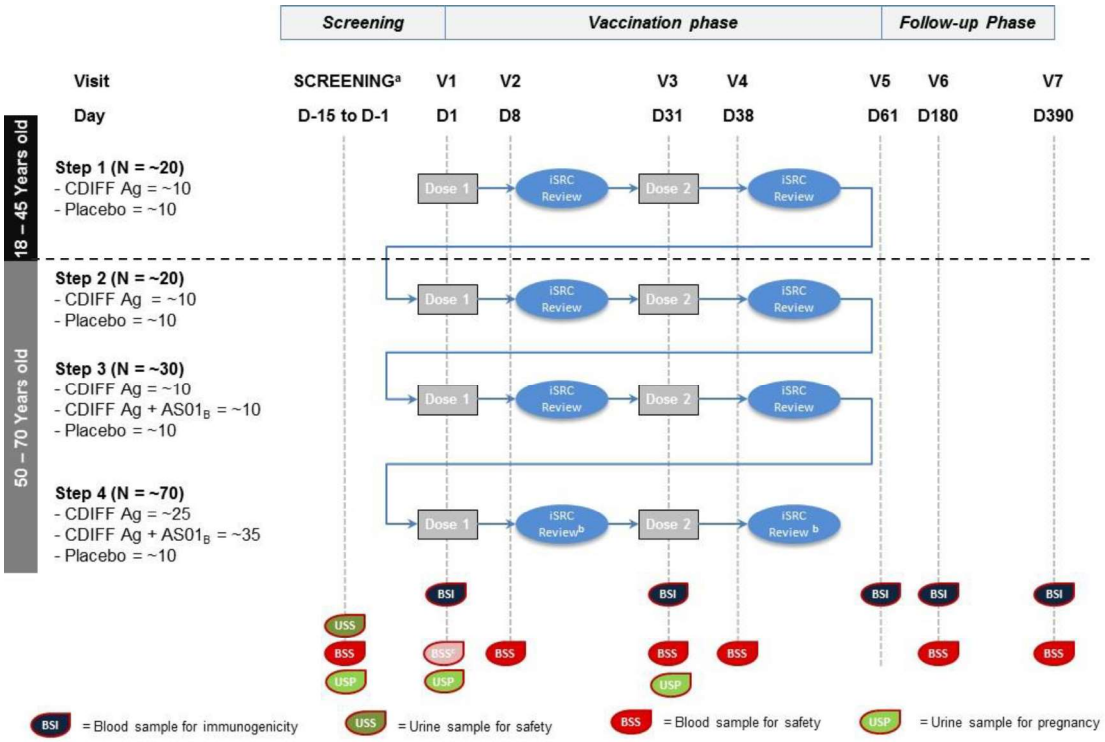
Study treatment will be administered as indicated below:

Type of Contact and Timepoint	Study Group	Treatment Name	Volume to be Administered	Route	Injection Site	
					Location	Laterality[a]
Visit 1 (Day 1)	Placebo	Placebo	0.5 mL	IM	Deltoid	Nondominant
	CDIFF Ag	CDIFF Ag	0.5 mL	IM	Deltoid	Nondominant
	CDIFF Ag + $AS01_B$	CDIFF Ag + $AS01_B$	0.5 mL	IM	Deltoid	Nondominant
Visit 3 (Day 31)	Placebo	Placebo	0.5 mL	IM	Deltoid	Nondominant
	CDIFF Ag	CDIFF Ag	0.5 mL	IM	Deltoid	Nondominant
	CDIFF Ag + $AS01_B$	CDIFF Ag + $AS01_B$	0.5 mL	IM	Deltoid	Nondominant
Visit 8 for Step 4 (Day 491)	CDIFF Ag	CDIFF Ag	0.5 mL	IM	Deltoid	Nondominant
	CDIFF Ag + $AS01_B$	CDIFF Ag + $AS01_B$	0.5 mL	IM	Deltoid	Nondominant

Ag = antigen; $AS01_B$ = an adjuvant system containing MPL, QS-21 and liposome (50 µg MPL and 50 µg QS-21); IM = intramuscular; MPL = 3-O-desacyl-4'-monophosphoryl lipid A.

[a] The nondominant arm is the preferred arm of injection. In case it is not possible to administer the vaccine in the nondominant arm, an injection in the dominant arm may be performed.

Table A: Study Design Overview



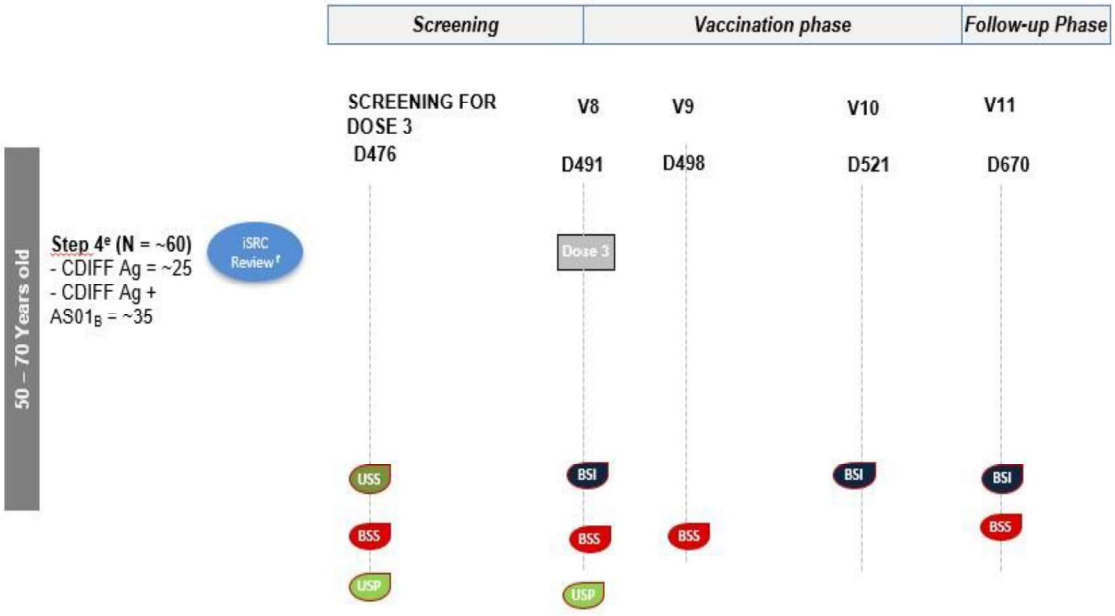


Table B: Intervals between Study Visits

Interval	Optimal Length of Interval	Allowed Interval
Screening → Visit 1 (Day 1)	1 to 15 days	-
Visit 1 (Day 1) → Visit 2 (Day 8)	7 days	7 to 9 days
Visit 1 (Day 1) → Visit 3 (Day 31)	30 days	30 to 35 days
Visit 3 (Day 31) → Visit 4 (Day 38)	7 days	7 to 9 days
Visit 3 (Day 31) → Visit 5 (Day 61)	30 days	30 to 35 days
Visit 3 (Day 31) → Visit 6 (Day 180)	149 days	140 to 160 days
Visit 3 (Day 31) → Visit 7 (Day 390)	359 days	350 to 395 days
Visit 7 (Day 390) → Screening Dose 3 (Day 476)	20 to 150 days	-
Screening Dose 3 (Day 476) → Visit 8 (Day 491)	1 to 15 days	-

Visit 8 (Day 491) → Visit 9 (Day 498)	7 days	7 to 9 days
Visit 8 (Day 491) → Visit 10 (Day 521)	30 days	30 to 35 days
Visit 8 (Day 491) → Visit 11 (Day 670)	179 days	170 to 190 days

3.2. SCHEDULE OF EVENTS

Schedule of events can be found in Section 1.3, Table 5 of the protocol.

3.3. CHANGES TO ANALYSIS FROM PROTOCOL

Given the staggered enrolment, step is used as stratification factor in the randomization algorithm. Step will be used as a covariate in the inferential analysis.

The sponsor has decided not to perform ELISA assay in the study. All references to concentrations from ELISA have been deleted from the last approved SAP in this document.

3.4. TREATMENT ALLOCATION TO THE SUBJECT

Treatment numbers will be allocated by dose.

The enrolment will be performed to control the distribution of the population within the age strata (subjects 18-45 years of age versus subjects 50-70 years of age) and within each step (step 1 to step 4).

Allocation of the subject to a study group at the study center will be performed using SBIR (a randomization system on internet). Within the 18-45 years age and step 1 strata, the randomization algorithm will use a minimization procedure accounting for gender (female, male), while within the 50-70 years age and step 2 to step 4 strata, the randomization algorithm will use a minimization procedure accounting for age (50-59 years of age, 60-70 years of age) and gender (female, male). Minimization factors will have equal weight in the minimization algorithm.

After obtaining the signed or witnessed/thumb printed and dated informed consent form (ICF) from the subject and having checked the eligibility of the subject, the study staff in charge of the vaccine administration will access SBIR. At each step, upon providing the age (subjects18-45 years of age versus subjects 50-70 years of age), the gender, and the subject identification number, the randomization system will determine the study group and will provide the treatment number to be used for the first dose.

The number of each administered treatment must be recorded in the eCRF on the Vaccine Administration screen. When SBIR is not available, please refer to the SBIR user guide or the study procedures manual (SPM) for specific instructions.

4. PLANNED ANALYSES

4.1. INTERNAL SAFETY REVIEW COMMITTEE (iSRC)

As the investigational vaccine formulation will be administered to human for the first time, an iSRC will be appointed. This study will be overseen by an iSRC operating under a charter. Please refer to iSRC charter for details.

4.2. SAFETY REVIEW TEAM (SRT)

The SRT is responsible for ongoing safety monitoring of the entire project and meets on a regular basis. The SRT will inform the iSRC about any potential safety concern relevant to the study (in a blinded way).

Before each iSRC safety evaluation in this study (see above), the SRT will review the same safety data, but in a blinded manner to keep all people involved in the conduct, cleaning and final analysis of the study blinded. Please refer to iSRC charter for details.

4.3. INTERIM ANALYSIS

A first analysis will be performed when all data up to Day 61 (ie, data that are as clean as possible) are available. The immunogenicity data available until Day 61, and the safety and reactogenicity data available at the time of this analysis will be summarized based on unblinded treatment groups. At this point, the IQVIA statistical team, including those responsible for creating the programs to produce the outputs for the Interim Analysis, will be unblinded for the analysis (ie, will have access to individual subject treatment assignments). Whenever it is needed after the interim analyses, the GSK statistical team may have access to individual subject treatment and data.

The remaining study personnel will stay blinded (ie, will not have access to the individual subject treatment assignment) until study end. It is possible however, due to the limited sample size, that unblinding occurs for a few subjects having a specific AE or SAE (eg, an AE/SAE occurring only in a single group). Therefore, anyone having access to the analysis of Day 61 could become unblinded regarding those specific cases. The study will be considered as partial-blind from this point onwards. Subjects will be provided with information about the study arm, via the investigator at the study end.

Note: In a partial-blind study, the subject, the investigator, or anyone assessing the outcome is aware

of the assignment in at least one treatment group but unaware of the treatment assignment(s) in one or more other treatment groups. Individual listings will be provided at the study end to the investigator.

The second interim analysis will be performed when all data up to 1 month after Dose 3, ie, Day 521 (ie, data that are as clean as possible) are available. The immunogenicity data available until Day 521, and the safety and reactogenicity data available at the time of this analysis will be described. No individual listings will be provided.

Given the multitude of the immunogenicity assays (TNA with different cell lines), the interim analysis may be performed in several parts upon the availability of assay results (until Day 61 or Day 521). The first part will include the available immunogenicity assay results (until Day 61 or Day 521) and safety data available. The subsequent parts will include only additional data results of immunogenicity assays that become available later. Any immunogenicity data (until Day 61 or Day 521) that become available at the end of the study will be included in the final (end of study) analysis.

All analyses are descriptive and with the aim to characterize the safety and immunogenicity data and therefore no statistical adjustment for interim analysis is required.

Derivations and definitions for the interim analyses will be based on those required for the final analysis contained in this analysis plan, unless deviations are stated within the text.

All tables presenting summary statistics planned for final analysis will be generated at the time of interim analyses, only for the primary cohort (exposed set for safety and per protocol set/full analysis set for immunogenicity analyses). No unblinded individual subject listings and listing tables will be generated at the time of interim analyses.

CCI [redacted]

4.4. FINAL ANALYSIS

All final, planned analyses identified in this SAP will be performed by IQVIA Biostatistics following sponsor authorization of this Statistical Analysis Plan, database lock, and unblinding of treatment. The final analysis will be performed when all data up to study end are available.

An integrated (final) CSR containing all data will be written and made available to the Principal Investigator at that time. The final CSR will contain at least the final analyses of all primary and secondary endpoints. In addition, all previous analyses will be re-produced based on cleaned data at this point. *Individual listings will only be provided at this stage*.

5. ANALYSIS SETS

5.1. ENROLLED SET

The enrolled set will contain all eligible subjects who provide informed consent for this study. Subjects in this population will be used for disposition summaries.

5.2. EXPOSED SET

The exposed set will contain all subjects who have received at least 1 dose of study treatment and will be analyzed according to treatment received. Subjects with invalid informed consent or fraudulent data will be excluded from this cohort. Subjects in this population will be used for demographic and dosing summaries.

The allocation in a group is done in function of the first administered treatment:

5.2.1. UNSOLICITED SAFETY SET

The unsolicited safety set will contain all subjects who have received at least 1 dose of study treatment (included in the Exposed Set) that report unsolicited AEs or report not having unsolicited AEs.

5.2.2. SOLICITED SAFETY SET

The solicited safety set will contain all subjects who received at least 1 dose of the study treatment (included in the Exposed Set) who have solicited safety data.

5.2.3. SUBCOHORT EXPOSED SET

The subcohort exposed set will contain all subjects who have received the Dose 3 on an active arm.

5.3. FULL ANALYSIS SET

The full analysis set will contain all subjects who received at least 1 dose of the study treatment to which they are randomized and have postvaccination immunogenicity data.

The allocation in a group is done in function of the first treatment as per randomization.

The Full Analysis Set will be performed if the percentage of vaccinated subjects with serological results excluded from the Per-protocol Set for analysis of immunogenicity is 10% or more.

5.4. PER-PROTOCOL SET

The per-protocol set will contain all subjects included in the full analysis set minus subjects with protocol deviations that lead to exclusion. The protocol deviations leading to exclusion of the subjects are described in Section 11.2. Three Per-protocol Sets for analysis of immunogenicity will be derived:

5.4.1. PER-PROTOCOL SET FOR ANALYSIS OF IMMUNOGENICITY AT DAY 61

The analysis set will contain all subjects included in the Per-protocol Set, with no protocol deviations leading to exclusion until Day 61 and with at least 1 immunogenicity data available at Day 61.

5.4.2. PER-PROTOCOL SET FOR ANALYSIS OF IMMUNOGENICITY AT DAY 180

The analysis set will contain all subjects included in the Per-protocol Set, with no protocol deviations leading to exclusion until Day 180 and with at least 1 immunogenicity data available at Day 180.

5.4.3. PER-PROTOCOL SET FOR ANALYSIS OF IMMUNOGENICITY AT DAY 390

The analysis set will contain all subjects included in the Per-protocol Set, with no protocol deviations leading to exclusion until study end (Day 390) and with at least 1 immunogenicity data available at Day 390.

5.5. SUBCOHORT PER-PROTOCOL SET

5.5.1. SUBCOHORT PER-PROTOCOL SET FOR ANALYSIS OF IMMUNOGENICITY AT DAY 521

The analysis set will contain all subjects included in the Per-protocol Set, who received the Dose 3 on an active arm, with no protocol deviations leading to exclusion until Day 521 and with at least 1 immunogenicity data available at Day 521.

5.5.2. SUBCOHORT PER-PROTOCOL SET FOR ANALYSIS OF IMMUNOGENICITY AT DAY 670

The analysis set will contain all subjects included in the Per-protocol Set, who received the Dose 3 on an active arm, with no protocol deviations leading to exclusion until Day 670 and with at least 1 immunogenicity data available at Day 670.

5.6. COVID-19 SET

The analysis set will contain all subjects included in the exposed set who were not able to go to the site for the Visit 5 (Day 61) on schedule, due to study being paused during COVID-19, and as a result, had their immunogenicity samples collected outside of the protocol specified interval. This analysis set will be used for additional descriptive summaries of immunogenicity variables.

Analysis	Enrolled Set	Exposed Set	Un-solicited Safety Set*	Solicited Safety Set	Full Analysis Set**	Per-protocol (PP) Set			Subcohort PP Set		COVID-19 Set
						PP Set Day 61	PP Set Day 180	PP Set Day 390	PP Set Day 521	PP Set Day 670	
Subject Disposition	✓										
Baseline Assessments		✓	✓			✓	✓	✓	✓	✓	
Concomitant Medications		✓									
Exposure		✓									
Immunogenicity Analysis					✓	✓	✓	✓	✓	✓	✓
Protocol Deviations		✓									
Adverse Events			✓	✓							
Other Safety (lab, vitals)		✓									

* The safety/reactogenicity analysis will be first performed on the exposed set. If >=10% of subjects have missing information for unsolicited symptoms, then the analyses on the unsolicited safety set will be performed.

** Analysis in this set will be performed if the percentage of vaccinated subjects with serological results excluded from the Per-protocol Set for analysis of immunogenicity is 10% or more

6. GENERAL CONSIDERATIONS

The safety summaries, data listings and figures as well as the statistical analysis of the variables will be the responsibility of the study biostatistician at IQVIA.

6.1. SUMMARY STATISTICS

For qualitative variables, the population size (N for sample size and n for available data) and the percentage (of available data) for each class of the variable will be presented. Quantitative variables will be summarized using descriptive statistics, including N (with data available), Nmiss (with missing data), mean, standard deviation (SD), coefficient of variation (CV%), median, Q1, Q3, minimum, and/or maximum values. Geometric mean will be included for immunogenicity parameters, where applicable. Coefficient of variation will not be presented for change from baseline results.

The exact two-sided 95% CIs for a proportion within a group will be calculated using the Clopper-Pearson exact method.

The geometric mean antibody titers (GMTs) calculations will be performed by taking the anti-log of the mean of the log10 titer transformations. Confidence interval for geometric means will be derived by raising 10 to the confidence interval associated with mean of log10 values i.e. CI of geometric mean = 10^(CI for the mean of log10 values). All subjects with data will be considered. Subjects whose antibody titers are below the cut-off of the assay will be given an arbitrary value of half the cut-off for the purpose of GMT calculation.

6.2. TREATMENT SUMMARIZATION

In general, data will be presented for each treatment group:

Age	Treatment
50-70 Y	CDIFF Ag + $AS01_B$
	CDIFF Ag
	Placebo
18-45 Y	CDIFF Ag
	Placebo

Data for all study subjects combined will also be presented when appropriate. Analysis by age in older adult cohort will be performed by age strata (50-59 years old and 60-70 years old) for the descriptive immunogenicity analyses if at least 6 subjects are available in these subgroups and in at least one active arm.

6.3. PRECISION

Safety data (vital signs, laboratory parameters) and immunogenicity data will be reported to the same

precision as the source data.

For the reporting of descriptive statistics, the mean, median, Q1, Q3, standard deviation, standard error and confidence intervals will be presented to one digit more precision than the source data. The minimum and maximum will be presented to the same precision as the source data. Coefficient of variation (%) will always be reported to 1 decimal place. P-values, if any, shall be reported to four decimal places or as <0.0001.

The number of decimals used when displaying geometric mean titers (GMT) and their confidence limits is shown in the following table:

GMT value	Number of decimals to display
<0.1	3
>=0.1 and <10	2
>=10 and <1000	1
>=1000	0

When multiple categories of GMT/GMC values are present in the same table, the number of decimals displayed should match that of the smallest category (i.e. the one with the higher number of decimals). GMFR or GMT/GMC ratios and their confidence limits will be displayed with 2 decimals regardless of the actual values.

6.4. REFERENCE START DATE AND STUDY DAY

Study Day will be calculated from the reference start date and will be used to show start/stop day of assessments and events.

Reference start date is defined as the day of the first dose of study vaccination, (Day 1 is the day of the first dose of study treatment) and will appear in every listing where an assessment date or event date appears.

- If the date of the event is on or after the reference date, then:

  Study Day = (date of event – reference date) + 1.

- If the date of the event is prior to the reference date, then:

  Study Day = (date of event – reference date).

In the situation where the event date is partial or missing, Study Day, and any corresponding durations will appear partial or missing in the listings. Refer to Section 7.1.1 for rules applicable on partially completed dates when used in calculations.

6.4.1. ATTRIBUTING EVENTS TO VACCINE DOSES

The dose relative to an event is the most recent study dose given to a subject 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 CRF using the contents of the flag indicating if the event occurred before or after vaccination. If 'after vaccination' is selected, the relative dose for the event will be the one administered on the start day of the event. If 'before vaccination' is selected, the relative dose for the event will be the dose prior to this one. Where applicable, the event will be classified as "pre-treatment".

6.5. BASELINE

Baseline is defined as the last scheduled non-missing measurement taken prior to dosing and will correspond to Day 1, predose for immunogenicity assessments, hematology and biochemistry, and vital signs. However, if a subject is missing the planned baseline result, the previous non-missing evaluation will become the baseline value.

6.6. COMMON CALCULATIONS

For quantitative safety measurements (e.g. vital signs, laboratory parameters), change from baseline will be calculated as:

- Test Value (after baseline) – Baseline Value

For immunogenicity measurements, change from baseline will be calculated as:

- Test Value (after baseline)/Baseline Value

6.7. SOFTWARE VERSIONS

All derivations, statistical analyses, summaries, graphs and listings will be generated using SAS version 9.4 or higher (SAS Institute, Inc., Cary, North Carolina).

7. STATISTICAL CONSIDERATIONS

7.1. MISSING DATA

Missing immunogenicity data will be handled as described in Section 15. Following imputation will be used for statistical calculations (and not for subject listings):

7.1.1. DATES

When partially completed birth dates (i.e. with missing day 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 vaccination) will be used to complete the date. If 'after vaccination' is selected, the imputed start date will match the first (or only) study dose given during that month. If 'before vaccination' 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 vaccination) will be used to complete the date. If 'after vaccination' is selected, the imputed start date will match the first (or only) study dose given during that year. If 'before vaccination' is selected, the imputed date will be one day before the first (or only) study dose given during that year.

All other cases of incomplete AE or concomitant medication/vaccination start date will follow the standard rules as outlined in Section 6.4.

7.1.2. LABORATORY DATA

Missing laboratory results will not be replaced.

7.1.3. ADVERSE EVENTS

Solicited Adverse Events:

Daily recording of solicited adverse events: For symptom screens indicating the presence or absence of solicited adverse events, the following rules are applicable:

- Denominators for the summary of local (or general) solicited adverse events will be calculated using the number of subjects who respond "Yes" or "No" to the question concerning the occurrence of local (or general) adverse events.

- When a specific solicited adverse event is marked as having not occurred following a specific vaccination (i.e. SDTM CE.CEOCCUR=N for the specified post-vaccination period for the adverse event in question), all daily measurements will be imputed as Grade 0.

- When a specific solicited adverse event is marked as having occurred following a specific vaccination (i.e. SDTM CE.CEOCCUR=Y for the specified post-vaccination period for the adverse 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 adverse event summary tables.

- When the occurrence of a specific solicited adverse event is not present (i.e. SDTM CE.CEOCCUR is neither Y nor N for the specified post-vaccination period for the symptom in question) but the group of solicited adverse events (local or general) 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 adverse event summary tables.

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

Solicited adverse event category	**Subjects included in the calculation of the numerator**
Any	All subjects 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 subjects 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 subjects 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 subjects with at least one occurrence of the adverse event at grade 3 between Day X and Day Y

Unsolicited adverse events

Unsolicited adverse event 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' in all statistical listings.

7.2. DATA DERIVATION

7.2.1. BODY MASS INDEX

BMI (kg/ m2) = weight (kg)/ height (m)2

7.2.2. AGE AT VACCINATION IN YEARS

When age at vaccination is to be displayed in years, it will be calculated as the number of complete calendar years between the date of birth and the date of vaccination. For example:

DOB = 10SEP1983, Date of vaccination = 09SEP2018 -> Age = 34 years

DOB = 10SEP1983, Date of vaccination = 10SEP2018 -> Age = 35 years

7.2.3. ONSET DAY

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

7.2.4. DURATION OF EVENTS

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

The duration of solicited events will be calculated as the sum of the individual days with the symptom reported at grade 1 or higher.

7.2.5. COUNTING RULES FOR COMBINING SOLICITED AND UNSOLICITED ADVERSE EVENTS

For output combining solicited and unsolicited adverse events, all serious adverse events will be

considered general events since the administration site flag is not included in the expedited adverse event CRF pages.

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

7.2.6. COUNTING RULES FOR OCCURRENCES OF SOLICITED ADVERSE EVENTS

When the occurrences of solicited adverse 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.

For a given subject and the analysis of solicited symptoms within 7 days post-vaccination, missing or non-evaluable measurements will not be replaced. Therefore the analysis of the solicited will include only vaccinated subjects for doses with documented safety data (i.e., symptom screen completed). More specifically the following rules will be used:

- Subjects who documented the absence of a solicited symptom after one dose will be considered not having that symptom after that dose.
- Subjects who documented the presence of a solicited symptom and fully or partially recorded daily measurement over the solicited period will be included in the summaries at that dose and classified according to their maximum observed daily recording over the solicited period.
- Subjects who documented the presence of a solicited symptom after one dose without having recorded any daily measurement will be assigned to the lowest intensity category at that dose (i.e. 38°C for fever or grade 1 for other symptoms).
- Doses without symptom sheets documented will be excluded.

7.2.7. GEOMETRIC MEAN TITERS (GMTS)

Geometric Mean Titer (GMT) calculations are performed by taking the inverse logarithm of the mean of the log titer transformations. Antibody titers below the cut-off of the assay will be given an arbitrary value of half the cut-off of the assay for the purpose of GMT calculation. The cut-off value will be defined by the laboratory before the analysis.

8. OUTPUT PRESENTATIONS

The templates provided with this SAP describe the presentations for this study and therefore the format and content of the summary tables, figures and listings to be provided by IQVIA Biostatistics.

Some minor modifications may be necessary to the planned design of tables, figures, and listings to accommodate data collected during the actual study conduct.

9. DISPOSITION AND WITHDRAWALS

All subjects who provide informed consent will be accounted for in this study. Subject disposition will be tabulated for each study treatment and for all subjects combined with the number of subjects who are randomly assigned to treatment, complete the study, prematurely discontinue, and the reason for early discontinuation. A listing will present dates of completion or early withdrawal and the reason for early discontinuation, if applicable, for each subject.

Listings of inclusion/exclusion criteria responses, study protocol deviations, treatment randomization and study treatment administration will be provided.

A listing of subjects whose trial participation was impacted by COVID-19 will be presented along with the description of the impact. Number of subjects who discontinued study drug or study due to COVID-19 infection or issues related to the COVID-19 pandemic will be summarized.

10. DEMOGRAPHIC AND OTHER BASELINE CHARACTERISTICS

Individual subject demographics and baseline characteristics (medical history and results from pregnancy tests) will be presented in listings.

Demographic characteristics such as age, sex, race, ethnicity, height, weight, and body mass index (BMI) will be summarized and tabulated by treatment and for all subjects overall. Descriptive statistics (mean, median and standard deviation) will be presented for age, height, weight, and BMI. Frequency counts and percentages will be presented for sex, race, ethnicity, age group (50-59 years old and 60-70 years old) and step.

No statistical testing will be carried out for demographic or other baseline characteristics.

11. PROTOCOL DEVIATIONS

11.1. DEVIATIONS RELATED TO STUDY CONDUCT

A protocol deviation is any noncompliance with the clinical trial protocol, good clinical practice (GCP), or Manual of Procedure requirements. The noncompliance may be either on the part of the subject, the site PI, or the study site staff.

Any subject enrolled who does not meet eligibility criteria will be considered an enrollment deviation. Protocol deviations will be listed including a classification of minor, major, or critical as determined by clinical staff. Any protocol deviation related to study conduct that is assessed as COVID-19 related will be clearly identified in the outputs and clinical study report.

11.2. DEVIATIONS RELATED TO IMMUNOGENICITY ANALYSIS

Changes to the procedures or events, which may impact the quality of the immunogenicity data, will be considered significant protocol deviations and will be described within the clinical study report. These changes or events will include any circumstances that will alter the evaluation of the immunogenicity results. Examples include, but may not be limited to, sample processing errors that lead to inaccurate immunogenicity results, and/or inaccurate dosing.

Subjects may be eliminated from the per-protocol Set for analysis of immunogenicity if, during the study, they incur a condition that has the capability of altering their immune response or are confirmed to have an alteration of their initial immune status. Subjects may also be eliminated from the per-protocol set based on usage of certain concomitant medications/products/vaccines as described in Section 6.8.2 of the protocol. Such scenarios will be discussed by the study team and decision to exclude a subject and/or data from the Per-protocol analysis set (Day 61, Day 180 and Day 390) or subcohort per-protocol analysis set (Day 521 and Day 670) will be taken on a case-by-case basis.

Other deviations (not a comprehensive list) leading to elimination from the per-protocol set are:

- Invalid informed consent
- Fraudulent data
- randomization code broken
- dose volume insufficient
- vaccination not according to protocol
- vaccine dose storage conditions (temperature deviation)
- vaccine administered out of expiry date
- violation of inclusion/exclusion criteria
- interval between doses
- interval between post vaccination blood sampling and previous dose
- two doses not in the same treatment group
- concomitant infection related to study vaccine or not related to study vaccine that may alter the immune response
- serology result at post vaccination dose 2 not available for all assays (blood sample not available or result not available)

Protocol Deviation Management Plan (PDMP) describes the complete list of elimination codes that could lead to exclusion from the per-protocol set.

12. MEDICAL HISTORY

Medical History coded using MedDRA Version 22.0 will be presented for the exposed set. Medical history for subjects assessed as probable, confirmed or suspected case of COVID-19 will be summarized.

13. MEDICATIONS

Medication usage coded using the GSK Drug Dictionary Version 1.6 will be presented for the exposed set.

- 'Prior' medications are medications which started and stopped prior to the first dose of study treatment.
- 'Concomitant' medications are medications which were taken during the treatment period, or specifically:
  - started after the first dose of study treatment or
  - started prior to the first dose of study medication and were continued after the first dose of study treatment

The percentage of subjects using concomitant medications (any medication, any antipyretic and any antipyretic taken prophylactically, respectively) during the 7-day follow-up period and during the 30-day follow-up period will be summarized by group after each vaccination and overall.

14. STUDY MEDICATION EXPOSURE

Exposure to study treatment as the number of doses administered will be presented for each treatment. Exposure for subjects assessed as probable, confirmed or suspected case of COVID-19 will be summarized separately.

15. IMMUNOGENICITY ANALYSIS

The primary analysis will be based on the Per-protocol Set for analysis of immunogenicity. If, in any study group, the percentage of vaccinated subjects with serological results excluded from the Per-protocol Set for analysis of immunogenicity is 10% or more, a second analysis based on the Full Analysis Set will be performed.

The analysis of subjects receiving Dose 3 on an active arm will be based on the Subcohort Per-protocol Set for analysis of immunogenicity.

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
"< 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

The following immunogenicity variables will be assessed:

- Anti-Toxin A neutralizing antibody titers (using HT29 cell line)
- Anti-Toxin B neutralizing antibody titers (using HCT116 cell line)

A listing of collection dates and times for the immunogenicity variables will be presented for subjects within each treatment group. For each test method, the neutralizing antibody titers from TNA assay will be listed and summarized by treatment groups across all scheduled timepoints during entire study.

For each study group, each age group (18-45 Y versus 50-70 Y) and/or subcohort (as applicable), at each timepoint that blood samples are collected for humoral immune response and for each assay:

- Percentage of subjects above the assay cutoff with their exact 95% CI will be tabulated
- Geometric mean titers and their 95% CI will be calculated
- Geometric mean fold-rise with their exact 95% CI will be tabulated
- Percentage of subjects with >=2, 4, 8, 16, 32, 64 fold-rise from baseline for each assay
- Percentage of subjects with >=2, 4, 8, 16, 32, 64 fold-rise from baseline for both anti-Tox A and anti-Tox B for neutralizing titers
- Antibody titers will be investigated using Reverse Cumulative Curves

The same tabulations will be performed by age 50-59 Y versus 60-70 Y and by serostatus at baseline (above or equal to cut-off versus below the cut-off) [if at least 6 subjects are available in these sub-group and in at least one active arm]

Linear mixed model with repeated measures will be used to perform pairwise comparison between groups in anti-toxin A neutralizing antibody titers and anti-toxin B neutralizing antibody titers at Day

31, Day 61, Day 180 and Day 390. The model will use fixed effects for treatment, time point, step, treatment×time point, baseline value as covariate, and a repeated time point effect within a subject under a unstructured covariance matrix. Variance between the groups will not be considered equal. Age and gender will be included in the model as covariates if the data allow. Additional covariance structures and suitable transformation (e.g. log) may be explored to attain convergence and meet model assumptions, respectively. This analysis will be performed on the age stratum 50-70 year old adults.

A similar linear mixed model will be used to evaluate the difference between treatments for subjects who received the 3rd dose except the model will not include a fixed effect for step. CCI [redacted]

Descriptive summaries planned for immunogenicity variables (titers) described above will additionally be presented for COVID-19 analysis set.

15.1. EXPLORATORY ANALYSIS

CCI [redacted]

16. SAFETY OUTCOMES

There will be no statistical comparisons between the treatment groups for safety data, unless otherwise specified with the relevant section.

16.1. ADVERSE EVENTS

Adverse Events (AEs) will be coded using Medical Dictionary for Regulatory Activities (MedDRA) central

coding dictionary, Version 22.0.

The percentage of subjects with at least 1 local AE (solicited and unsolicited), with at least 1 general AE (solicited and unsolicited) and with any AE during the 7-day or the 30-day follow-up period will be tabulated with exact 95% CI after each vaccination and overall.

The percentage of doses followed by at least 1 local AE (solicited and unsolicited), by at least 1 general AE (solicited and unsolicited) and by any AE will be tabulated, overall vaccination course, with exact 95% CI. The same computations will be done for Grade 3, any AEs considered related to vaccination, any Grade 3 AEs considered related to vaccination and any AEs resulting in a medically attended visit.

The percentage of subjects/doses reporting each individual solicited local and general AE during the 7-day follow-up period will be tabulated with exact 95% CI as follows:

- Over the 2 or 3 doses, the percentage of subjects with the symptom and its exact 95% CI.
- Over the 2 or 3 doses, the percentage of doses with the symptom and its exact 95% CI.
- At each study dose (visit), the percentage of subjects with the symptom and its exact 95% CI.

Occurrence of fever will be reported per 0.5°C cumulative increments as from ≥38°C as well as the occurrence of fever >40°C/104°F with causal relationship to the study treatment.

The verbatim reports of unsolicited symptoms will be reviewed by a physician and the signs and symptoms will be coded per the Medical Dictionary for Regulatory Activities (MedDRA). Every verbatim term will be matched with the appropriate preferred term. The percentage of subjects with unsolicited AEs within 30 days after any doses with its exact 95% CI will be tabulated by group and by MedDRA preferred term. Similar tabulation will be done for Grade 3 unsolicited AEs, for unsolicited AEs that resulted in a medically attended visit, for Grade 3 and causally related unsolicited AEs and for unsolicited AEs causally related to vaccination. These tabulations will include SOC and SOC/PT analyses.

The number of subjects who experienced at least 1 SAE or any AE of specific interest (potential immune mediated disease) within 30 days after any dose, from dose 1 until 30 days post last dose and during the entire study period will be reported.

The following summaries for COVID-19 infections will be presented:

- Number of subjects with an AE of suspected, probable or confirmed assessment of COVID-19 infection
- Number of subjects who had a COVID-19 diagnosis test performed and the number of subjects with positive, negative or indeterminate results
- Incidence of COVID-19 as AEs and SAEs
- Summary of severity, duration and outcome of AEs related to COVID-19
- Incidence of AEs over the course of trial (pre and during pandemic)
- Incidence of AEs by age and gender over the course of trial (pre and during pandemic)

Events observed on or after 01DEC2019 will be deemed to occur during the pandemic while the events observed prior to 01DEC2019 will be considered pre-pandemic.

16.1.1. Adverse Events Leading to Discontinuation from Study or from Treatment

Adverse events leading to permanent discontinuation from study will be identified by using the variable pertaining to outcome the Adverse Events page of the (e)CRF, and listed. AEs leading to discontinuation from treatment will be listed.

16.1.2. Serious Adverse Events

Serious adverse events (SAEs) are those events recorded as "Serious" on the Adverse Events page of the (e)CRF, and will be listed.

16.1.3. Derivation

Adverse Event	Intensity grade	Parameter
CCI - This section contained Clinical Outcome Assessment data collection questionnaires or indices, which are protected by third party copyright laws and therefore have been excluded.		

[a]Fever is defined as temperature ≥38.0°C/100.4°F

16.2. Safety Laboratory Evaluations

Laboratory results will be included in the reporting of this study for Hematology, Biochemistry and Urinalysis. A list of laboratory assessments to be included in the outputs is included in Appendix 4 of the protocol, Table 23.

For each group and for each haematology, biochemistry, and urinalysis parameter:

- The percentage of subjects having hematology, biochemistry, and urinalysis results below or above the local laboratory normal ranges will be tabulated by timepoint
- The maximum grading from Screening up to Visit 5 (Day 61)/ Visit 7 (Day 390) for subjects receiving 2 doses/Visit 11 (Day 670) for subjects receiving a third dose will be tabulated.
- Frequency of Grade 3/4 laboratory toxicities will be summarized for subjects with COVID-19 infection.
- Individual profiles for each hematology and biochemistry parameter will be plotted by treatment group for which FDA grading scale is defined.

16.2.1. GRADING FOR LABORATORY DATA

Grades will be based on local laboratory normal ranges and derived from Food and Drug Administration [FDA] Guidance to Industry "Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" [refer to Table 8 in protocol]). Those laboratory parameters not included in the FDA grading scale will not be graded).

16.3. VITAL SIGNS

The following Vital Signs measurements will be reported for this study:

- Resting systolic and diastolic blood Pressure (mmHg)
- Resting heart rate (bpm)
- Temperature (^{o}C)

The following summaries will be provided for vital signs data:

- Actual and change from baseline

16.4. COVID-19

Data collected on the COVID-19 Corona virus infection assessment page from the eCRF will be presented in a listing.

17. DATA NOT SUMMARIZED OR PRESENTED

The other variables not summarized or presented are:

- Cross-neutralization data generated post dose 2 and post dose 3

- Comments

These variables will not be summarized or presented, but will be available in the clinical study database, SDTM and/or ADaM datasets.

18. REFERENCES

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