STATISTICAL ANALYSIS PLAN

Study Title:	**A Phase 3, Randomized, Double-Blind, Controlled, Multicenter, Clinical Study to Evaluate Safety and Immunogenicity of an MF59-Adjuvanted Quadrivalent Subunit Influenza Vaccine in Comparison with an MF59-Adjuvanted Trivalent Subunit Influenza Vaccine and an MF59-Adjuvanted Trivalent Subunit Influenza Vaccine Containing the Alternate B Strain, in Adults Aged 65 Years and Above**	
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LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Events of Special Interest
aQIV	Adjuvanted Quadrivalent Influenza Vaccine
aTIV	Adjuvanted Trivalent Influenza Vaccine
BMI	Body Mass Index
CSR	Clinical Study Report
CBER	Center for Biologics Evaluation and Research
CI	Confidence Interval
CRF	Case Report Form
DMC	Data Monitoring Committee
FAS	Full Analysis Set
FDA	Food and Drug Administration
EDC	Electronic Data Capture
HI	Hemagglutination Inhibition
GMR	Geometric Mean Ratio
GMT	Geometric Mean Titer
GMTr	Geometric Mean Titer Ratio
ICF	Informed Consent Form
ID	Identification
ILI	Influenza-Like Illness
IRB	Institutional Review Board
IRT	Interactive Response Technology
MedDRA	Medical Dictionary for Regulatory Activities
MN	Microneutralization
NOCD	New Onset of Chronic Disease
NP	Nasopharyngeal
PD	Protocol Deviation
PPS	Per Protocol Set
PT	Preferred Term
RT-PCR	Reverse Transcription Polymerase Chain Reaction
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SC	Seroconversion
SCR	Seroconversion Rate
SD	Standard Deviation
SDA	Source Data Agreement
SOC	System Organ Class
SUSAR	Suspected Unexpected Serious Adverse Reactions
WHO	World Health Organization

1. BACKGROUND AND RATIONALE

This document presents the statistical analysis plan (SAP) for Seqirus, Protocol No. V118_18: A Phase 3, Randomized, Double-Blind, Controlled, Multicenter, Clinical Study to Evaluate Safety and Immunogenicity of an MF59-Adjuvanted Quadrivalent Subunit Influenza Vaccine in Comparison with an MF59-Adjuvanted Trivalent Subunit Influenza Vaccine and an MF59-Adjuvanted Trivalent Subunit Influenza Vaccine Containing the Alternate B Strain, in Adults Aged 65 Years and Above.

The aim of this study is to demonstrate that inclusion of an additional B vaccine strain elicits an immune response to the second B virus and does not adversely impact the patient's immunogenicity response against strains previously included in trivalent formulations. Additionally, the aim is to demonstrate and confirm an acceptable safety and tolerability profile for the quadrivalent influenza vaccine formulation in the elderly population.

It describes the data and variables to be summarized and analyzed, including specifics of the statistical analyses to be performed. This analysis plan is based on the version 1.0 protocol dated 18 May 2017 and is compliant with ICH Harmonized Tripartite Guideline, 5 February, 1998, Statistical Principles for Clinical Trials, E9; World Health Organization, WHO Technical Report, Series No. 924. 2004, Annex 1: Guidelines on Clinical Evaluation of Vaccines: Regulatory Expectations; and FDA Center for Biologics Evaluation and Research (CBER) Guidance for Industry, May 2007, Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines.

The SAP provides the description of the analysis for the active study period and safety data through to the final evaluation (6 months following last study vaccination dose). Data from this study will be used to support the licensure of adjuvanted quadrivalent influenza vaccine (aQIV) for the prevention of seasonal influenza in adults ≥ 65 years of age.

2. STUDY OBJECTIVES AND ENDPOINTS

2.1 PRIMARY OBJECTIVES AND ENDPOINTS

2.1.1 PRIMARY OBJECTIVES

The co-primary immunogenicity objectives are:

1. To demonstrate that vaccination with aQIV elicits an immune response that is not inferior to that of an adjuvanted trivalent influenza vaccine (aTIV) containing the same virus strains as the US licensed adjuvanted influenza vaccine (Fluad, aTIV-1), and an aTIV containing the alternate B strain (aTIV-2) among adults ≥ 65 years of age.

2. To assess the immunogenicity of aQIV in adults ≥ 65 years of age, based on the CBER recommendations.

2.1.2 CO-PRIMARY ENDPOINTS

The immunogenicity of study vaccines will be assessed 21 days (i.e., on Day 22) after vaccine administration by measuring the hemagglutination inhibition (HI) antibody titers to the virus strains included in the vaccines.

The non-inferiority of aQIV compared to aTIV-1, and to aTIV-2 will be assessed for the eight co-primary endpoints of HI geometric mean titer (GMT) and seroconversion rate (SCR) for each virus strain included in the vaccines as follows:

- The GMT ratio* for the A/H1N1 strain;
- The GMT ratio for the A/H3N2 strain;
- The GMT ratio for the B strain (Yamagata lineage);
- The GMT ratio for the B strain (Victoria lineage);
- The difference between the SCR**for the A/H1N1 strain;
- The difference between the SCR for the A/H3N2 strain;
- The difference between the SCR for the B strain (Yamagata lineage);
- The difference between the SCR for the B strain (Victoria lineage).

Immunogenicity results obtained from aTIV-1 and aTIV-2 for both A/H1N1 and A/H3N2 strains will be pooled for comparison with aQIV.

**The GMT ratio is defined as the geometric mean of the post-vaccination (Day 22) HI titer for aTIV-1 (or aTIV-2) over the geometric mean of post-vaccination (Day 22) HI titer for aQIV.*
***The SCR is defined as the percentage of subjects with either a pre-vaccination HI titer < 1:10 and a post-vaccination HI titer ≥ 1: 40 or a pre-vaccination HI titer ≥ 1:10 and a ≥ 4-fold increase in post-vaccination HI titer.*

The second co-primary immunogenicity objective for aQIV will be assessed 21 days after vaccine administration by applying CBER criteria for the elderly population for each of the 4 strains included in aQIV. The second co-primary endpoints include:

- The percent of subjects achieving seroconversion (SC) for HI antibody
- The percent of subjects achieving an HI antibody titer ≥ 1:40

The study is considered successful if all co-primary endpoints are achieved.

2.2 SECONDARY OBJECTIVES AND ENDPOINTS

2.2.1 SECONDARY IMMUNOGENICITY OBJECTIVES

The secondary objectives of the study are to assess the following, among adults aged ≥ 65 years:

1. To characterize the immunogenicity of aQIV, the aTIV-1 containing the same virus strains as the US licensed adjuvanted trivalent influenza vaccine, and the aTIV-2 containing the alternate B strain, by HI.
2. To demonstrate the immunological superiority of aQIV compared to aTIV-1 and aTIV-2 for the B strain that is not included in each TIV vaccine separately;

2.2.2 SECONDARY IMMUNOGENICITY ENDPOINTS

The secondary immunogenicity endpoints are:

- The measures of immunogenicity of aQIV, aTIV-1 and aTIV-2 used for Secondary objective 1 as determined by the HI assay against homologous strains at Day 1 and 22 (unless indicated otherwise), include the following:
  - GMT: Geometric mean of HI titers on Day 1(pre-vaccination) and Day 22 (post-vaccination);
  - Geometric mean ratio (GMR*): The geometric mean of the fold increase of post-vaccination HI titer over the pre-vaccination HI titer (Day 22/Day 1);
  - The percentage of subjects with a titer ≥1:40 at Day 1 and Day 22;
  - SCR: the percentage of subjects with either a pre-vaccination HI titer < 1:10 and a post-vaccination HI titer ≥ 1:40 or a pre-vaccination titer ≥ 1:10 and a ≥ 4-fold increase in post-vaccination titer.

* GMR is defined as the geometric mean of the fold increases of post-vaccination antibody titer over the pre-vaccination antibody titer.

- For Secondary objective 2, the immunologic superiority of the alternate B strain (e.g. the influenza B strain included in the aQIV but not in the aTIV formulation) in aQIV will be assessed for each aTIV separately, by using the co-primary endpoints of the ratio of HI GMT and the difference of SCR for each B virus strain 21 days after the last vaccination.

2.2.3 SECONDARY SAFETY OBJECTIVES

1. To assess safety and tolerability of aQIV, aTIV-1, and aTIV-2 among adults ≥ 65 years of age.

2.2.4 SECONDARY SAFETY ENDPOINTS

Safety and tolerability will be assessed by the frequency and severity of:

- Solicited local and systemic adverse events (AEs) for 7 days following vaccination (Day 1 through Day 7)
- All unsolicited AEs for 21 days following vaccination (Day 1 through Day 22) and medication associated with these events
- Serious AEs (SAEs), AEs leading to withdrawal from the study, new onset of chronic diseases (NOCDs), AEs of special interest (AESIs), and concomitant medications associated with these events as collected from Day 1 through Day 181.

2.3 EXPLORATORY OBJECTIVES

The exploratory immunogenicity objectives are:

1. To explore the association between HI immune response after administration of aQIV or the aTIV-1, containing the same virus strains as the US-licensed adjuvanted trivalent influenza vaccine, and the aTIV-2 containing the alternate B strain by baseline characteristics.
2. Additional optional objectives include characterization of the immunogenicity of aQIV using other immunological assays (e.g., virus neutralization [MN] or anti-neuraminidase antibody assays may be performed).

Both exploratory objectives may be performed using either homologous or heterologous strains, or both.

2.3.1 EXPLORATORY ENDPOINTS

The exploratory immunogenicity endpoints include:

- GMTs: Geometric mean HI titers (GMTs) at Day 1 and Day 22
- SCRs: Percentage of subjects with either a pre-vaccination HI titer < 1:10 and a post-vaccination HI titer ≥ 1:40, or a pre-vaccination titer ≥ 1:10 and a ≥ 4-fold increase in post-vaccination titer at Day 22.
- In case of any additional optional immunogenicity analyses, such as MN, or assessments using heterologous influenza strains, the immune response will be characterized in a similar manner as described in the secondary immunogenicity endpoints section above.

2.4 SUCCESS CRITERIA

The study is considered successful if both co-primary objectives are achieved.

2.4.1 Success Criteria for Co-Primary Endpoints

2.4.1.1 To Demonstrate Non-Inferiority

In line with the FDA Guidance on seasonal inactivated influenza vaccines (Guidance for Industry Clinical Data Needed to Support Licensure of Seasonal Inactivated Influenza Vaccines 2007), aQIV will be considered to be non-inferior to aTIV-1, containing the same virus strains as the US licensed adjuvanted trivalent influenza vaccine, and aTIV-2, containing the alternate B strain if, for each of the four strains, the following statistical criteria are met:

- The upper bound of the two-sided 95% confidence interval (CI) for the ratio of the GMTs does not exceed 1.5. The GMT ratio will be calculated as GMT_{aTIV}/GMT_{aQIV}; and

- The upper bound of the two-sided 95% CI for the difference between the SCRs does not exceed 10%. The difference in SCRs will be calculated as $SCR_{aTIV}–SCR_{aQIV}$

2.4.1.2 CBER Criteria for Co-Primary Endpoints:

The endpoints for percent of subjects vaccinated with aQIV achieving SC and HI titer ≥ 1:40 at Day 22 will be assessed against the criteria described in CBER Guidance Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines (FDA, 2007), namely

- The lower bound of the two-sided 95% confidence interval for the percentage of subjects achieving SC for HI antibody should meet or exceed 30%

- The lower bound of the two-sided 95% confidence interval for the percentage of subjects achieving a post-vaccination HI antibody titer ≥ 1:40 should meet or exceed 60%

The statistical evaluation consists of the observed proportion together with the lower bound of the corresponding two-sided 95% CI per strain. No adjustment for type I error for multiplicity will be made.

2.4.2 Success Criteria for Secondary Objectives

2.4.2.1 Success Criteria for Secondary Immunogenicity Endpoints

There are no specific success criteria for the assessment of immunogenicity results (first secondary objective) as the characterization of the immunogenicity of the vaccines are descriptive in nature.

Superiority of aQIV versus aTIV-1 and aTIV-2 for the alternate B strain will be assessed using the GMT ratio (GMT_{aTIV}/GMT_{aQIV}) and difference in SCR ($SCR_{aTIV} – SCR_{aQIV}$) at Day 22. Point estimates and two-sided 95% CIs will be obtained as described for the primary endpoint. Superiority will be declared if the upper limit of the two-sided 95% CI for the difference in ($SCR_{aTIV} – SCR_{aQIV}$) is < 0%, and the upper limit of the two-sided 95% CI for the GMT ratio (GMT_{aTIV} / GMT_{aQIV}) is < 1 for both B strains.

3. STUDY DESIGN

This phase 3 study is a randomized, double-blinded, comparator controlled, parallel-group, multicenter study of aQIV versus the US-licensed 2017-2018 adjuvanted trivalent influenza vaccine (aTIV-1, Fluad)**,** and versus an adjuvanted trivalent influenza vaccine (aTIV-2), containing the alternate B strain. All study vaccine administration will be a single dose, and consistent with the current license for aTIV-1.

The study will be conducted in approximately 1778 male and female adults aged 65 years and older who are healthy or have co-morbidities which increase their risk of complications from influenza infection. Subjects will be randomized to one of the three treatment groups in a 2:1:1 ratio. An Interactive Response Technology (IRT) system will be used for subject randomization.

Subjects will provide serological specimens during the two mandatory clinic visits before and after vaccination on Day 1 and Day 22, respectively, for measurement of immune responses in routine influenza assays. Each subject will complete a Diary Card for solicited local and systemic AEs for 7 days after vaccination. Safety information will also be collected for all unsolicited AEs for 21 days following vaccination; and for the full duration of study participation for serious AEs (SAEs), AEs leading to withdrawal from the study NOCDs, AESIs, and concomitant medications associated with these events. Three scripted safety telephone calls will facilitate the collection of these safety data during the study period, and one reminder phone call will occur during the first few days after vaccination to remind the subject on the completion of the Diary Card.

An unscheduled study visit may be performed at any time during the study to further evaluate safety information described on the telephone.

Any subject who manifests signs of an influenza-like illness (ILI) during the treatment period (Days 1-22) will be evaluated by real time reverse transcription polymerase chain reaction (RT-PCR) testing of a nasopharyngeal (NP) specimen for influenza. Subjects with onset of ILI and RT-PCR-confirmed influenza during the treatment period (Days 1-22) will be removed from the Per Protocol Set (PPS) in the immunogenicity analyses. ILI will also be recorded as an AE.

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

Table 3-1: Time and Events Table

Assessment	Clinic Visit 1	Reminder Phone Call	Safety Phone Call	Clinic Visit 2	Safety Phone Call	Safety Phone Call[12]
	Day 1	Day 3 (-1 to + 1 days)	Day 15 (-3 to + 3 days)	Day 22 (-3 to + 3 days)	Day 91 (-7 to +7 days)	Day 181 (-14 to + 14 days)
EDC Visit no:	1	2	3	4	5	6
Informed consent[1]	✓					
Demographics and influenza vaccination history[2,3]	✓					
Medical history and baseline medication use[3]	✓					
Physical examination [3,4]	✓			✓		
Oral temperature, vital signs, height, weight[3]	✓					
Review of eligibility criteria[3]	✓					
Blood sample for immunogenicity testing[3]	✓			✓		
Vaccination[5,6]	✓					
Provision of study supplies and instructions[7,8]	✓					
Subject Diary Card reviewed and collected				✓		
Telephone contact[9]		✓	✓		✓	✓
Assess influenza-like illness (*if applicable*)[10]	← ✓ →					

Assessment	Clinic Visit 1 Day 1	Reminder Phone Call Day 3 (-1 to + 1 days)	Safety Phone Call Day 15 (-3 to + 3 days)	Clinic Visit 2 Day 22 (-3 to + 3 days)	Safety Phone Call Day 91 (-7 to +7 days)	Safety Phone Call[12] Day 181 (-14 to + 14 days)
Assess all unsolicited AEs and concomitant medications[11]			✓	✓		
Assess SAEs, NOCDs, withdrawal AEs, AESIs & associated medications	←	←	←	✓	→	→

1. The invitation to participate and the informed consent process must be conducted within 10 days before the day of vaccination (Day 1)
2. Confirm that patient has had the opportunity to ask questions and consent form(s) are signed prior to any V118_20 procedures being performed.
3. Procedure to be performed prior to vaccination. All eligibility criteria must be met before any study procedures (eg., blood sampling) can be performed.
4. Physical examination must be performed by a qualified health care practitioner in accordance with local regulations and licensing requirements designated within the Site Responsibility Delegation Log.
5. Vaccination should occur only after the pre-dose blood sample collected.
6. After vaccination, the subject will remain under medical supervision for at least 30 minutes and observed for local and systemic AE and unsolicited AEs. A body temperature measurement (preferably oral) will be taken.
7. A Diary Card, thermometer and ruler will be dispensed at Day 1. Subject will receive instruction on diary completion and thermometer and ruler use.
8. On Day 1 approximately 6 hours after the vaccination or prior to going to bed at the latest, and daily thereafter through Day 7, solicited local and systemic AEs including other AEs (i.e., body temperature measurements and use of analgesics/antipyretics) will be reported daily by the subject in the Diary Card.
9. The reminder call at Day 3 will be made by site staff to remind the subject to complete the Diary Card. In the safety calls at Day 15, Day 91 and day 181, subjects will be interviewed by site staff using a scripted interview for collection of safety data. These safety data will be collected in source documents by the individuals performing the interviews.
10. The subject will be asked to contact the site staff if the subject experiences ILI symptoms[a] during the interval between Day 1 and Day 22 (i.e., up to and including the day of the Day 22 blood sample). The subject should visit the site within 3 days of the onset of the ILI in order for a nasopharyngeal swab sample to be taken, however samples will be accepted if collected up to 6 days following the day of ILI onset. The sample will be sent to the central laboratory for RT-PCR analysis.
[a] defined as at least one of the following respiratory symptoms: sore throat, cough, sputum production, wheezing, or difficulty breathing; concurrently with at least one of the following systemic symptoms: oral temperature of >37.2°C/ 99°F, chills, tiredness, headache, or myalgia.
11. All AEs through the day 22 visit, and medications used to treat them.
12. Any subject who terminates the study prematurely after receiving a vaccination should have a termination visit/call (Section 5.5.1).

4. RANDOMIZATION AND BLINDING

4.1 Randomization

Enrolled subjects will be randomized in the IRT system and automatically assigned a unique Subject ID. The Subject ID will be the subject's unique identification number for all CRFs and associated study documentation that will be used for duration of the study. After randomization, the Screening Number ceases to be used and remains in the Screening and Enrollment Log only. The list of randomization assignments is produced by the IRT service provider and approved by Seqirus or delegate.

Subjects will be randomized in a 2:1:1 ratio to aQIV, aTIV-1, and aTIV-2.

If for any reason, after signing the informed consent form (ICF), the subject who is eligible and enrolled fails to be randomized, this is called a randomization failure. Further guidance is provided in the CRF Instructions. The reason for all randomization failures should be recorded in the Screening and Enrollment Log and in the source document as specified in the Source Data Agreement (SDA). The information on subjects who are randomization failures should be kept distinct from subjects who are screen failures, as described in Section 5.1.2, Screening of the protocol.

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

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

4.1.1 Definition of Randomization/Vaccination Errors

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

- Subject was vaccinated with a vaccine different from the one assigned at randomization
- Subject was vaccinated with the correct vaccine but containing a lower volume

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

4.1.2 Forced Randomization

Forced randomization will not be utilized in this trial.

4.2 BLINDING AND UNBLINDING PROCEDURE

The trial is designed as a double-blind study. There are no visible differences between the investigational aQIV vaccine and the two comparator aTIV vaccines. Vaccines will be selected

and administered according to the Pack ID assigned to the subjects by IRT. Neither the subject nor any of the investigative staff who are involved in administering the vaccines or clinical evaluation of the subject will be aware of the vaccine administered.

In case of an emergency, the Investigator can determine the subject's assigned vaccine. The information can be retrieved from the IRT system either via web or phone (a 24/7 backup service).

Except in the case of medical necessity, a subject's treatment should not be unblinded without the approval of the Sponsor. In general, unblinding should only be performed when knowledge of the assigned treatment will affect a subject's management. In such instance of medical emergency, every effort should be made to contact the Sponsor prior to unblinding. If unblinding should occur (by either accidental unblinding or emergency unblinding for an SAE) prior to completion of the study, the Investigator must promptly contact the Sponsor and document the circumstances on the appropriate forms. Instructions regarding emergency unblinding will be provided to the Investigator.

All personnel involved in performing laboratory assays and others who are directly involved in the conduct of the trial or in the analysis of the final trial results will remain blinded to the treatment codes until at least the database has been locked for final analysis.

For details please refer to section 3.3 of the protocol.

If a subject is unblinded during the study, it is to be reported as major PD, except for subjects unblinded by Pharmacovigilance due to suspected unexpected serious adverse reactions (SUSAR). The unblinding will be documented appropriately *(for details see SOP on blinding)*.

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

5. SAMPLE SIZE AND POWER CONSIDERATIONS

aQIV will be tested against aTIV comparators. The treatment randomization ratio is 2:1:1 (aQIV: aTIV-1: aTIV-2). This study is powered to achieve 80% power to demonstrate noninferiority over 8 co-primary endpoints, SCRs for 4 strains, GMT for 4 strains using a one-sided alpha of 0.025 for each comparison. No adjustment for multiple endpoints will be made.

For comparisons of SCR a noninferiority margin of 10% (aTIV – aQIV) will be employed. It is assumed that the SCRs for A/ H1N1, A/ H3N2 and B strains for TIV are 73%, 73% and 40% respectively. These estimates are based on the estimated SCR rates of historical data, namely study protocol V70_27. It has been assumed that there is no difference in terms of SCR between aQIV and aTIV for all strains. For comparison of the GMT ratio, a noninferiority of 1.5 (aTIV/aQIV) will be employed. It is assumed that there is no difference between aQIV and aTIV (i.e., a ratio of 1) and that the standard deviation (SD) of log (titer) is 1.2.

Under these assumptions, N FAS = 800 in the aQIV group and 400 in each aTIV group providing 800 and 800 subjects receiving aQIV and aTIV respectively for comparisons of A strains, and 800 and 400 subjects receiving aQIV and aTIV respectively for comparisons of B strains. This provides a total N FAS = 1600. These numbers provide 99.45% power to detect differences in SCR for each A strain and 91.29 % power for each B strain, providing overall 82.42% power for the 4 SCR tests.

For GMT ratio tests each A strain test will have 100% power and each B strain test will have 99.98% power, providing 99.96% power for the 4 GMT ratio tests and consequently 82.39% power for the 8 co-primary endpoints. Overall 1600 FAS subjects will be required. Allowing for a 10% drop-out N=1778 subjects will be recruited.

The immunogenicity was powered to meet the criteria pre-specified in the CBER guidance for acceptable immunogenicity: namely,

- the lower bound of the two-sided 95% confidence interval for the percent of subjects achieving SC should exceed 30%, and
- the lower bound of the two-sided 95% confidence interval for the percent of subject achieving HI antibody titer ≥ 1:40 should exceed 60%

With a sample size of N=800 (FAS) subjects for the aQIV group if the population SCR for A/ H1N1, A/ H3N2 and B strains for TIV is 73%, 73% and 40% respectively then the probability of observing a SCR that is significantly greater than 30% is approximately 100% (for the A/H1N1), 100%for A/H3N2 and 100% (for the B-strains).

With a sample size of N=800 (FAS) subjects for the aQIV group if the percent of subject achieving HI antibody titer ≥ 1:40 for A/ H1N1, A/ H3N2 and B strains for TIV is 91%, 99% and 64.6% respectively (as observed in V70_27) then the probability of observing a SP rate that is significantly greater than 60% is approximately 100% (for the A/H1N1), 100% for A/H3N2 and 76.47% (for the B-strains).

Table 5-1 summarizes the list of all co-primary endpoints with strains, the planned noninferiority margin and underlying assumptions used for the sample size computations.

Table 5-1: Summary of Assumptions Used for Sample Size Calculations

NI comparison	**H1N1**	**H3N2**	**B strains**
Test significance level, alpha (1-sided)	2.50%	2.50%	2.50%
Noninferiority Margin for the SCR comparison (%)	10	10	10
Assumed true SCR	73%	73%	40%
Power for SCR comparison tests for each strain (%)	99.45%	99.45%	91.29%
Power for SCR comparison tests for each strain (%)	99.45%	99.45%	91.29%
Global Power for 4 SCR Endpoints	**82.42%**		
Noninferiority Margin for the GMT ratio	1.5	1.5	1.5
Common Standard Deviation of $\log_e$(titer)	1.2	1.2	1.2
Power for GMT ratio tests for each strain (%)	99.99%	99.99%	99.98%
Global Power for 4 GMT ratio Endpoints	**99.96%**		
Global Power for 8 Co-primary Endpoints	**82.39%**		
CBER Criteria	**H1N1**	**H3N2**	**B strains**
Test significance level, alpha (1-sided)	2.50%	2.50%	2.50%
Seroconversion Threshold	30%	30%	30%
Assumed true SCR	73%	73%	40%
Power for SCR	100%	100%	100%
Global Power for 4 SCR Endpoints	**100%**		

Threshold for HI titres ≥ 1:40	60%	60%	60%
Assumed True percent of subject achieving HI antibody titer ≥ 40	91%	99%	64.6%
Power for percent of subject achieving HI antibody titer ≥ 40≥ 1:40	100%	100%	76.47%
Global Power for all 4 percents of subject achieving HI antibody titer ≥ 40e	**76.47%**		
Global Power for achieving the (CBER Criteria)	**76.47%**		

A safety database of N = 800 has a 95% chance of detecting AEs that occur at a rate of 1 in 267. With a single stratum of 800 participants, the probability of observing at least one event for events with population rates of 1 in 300, 1 in 200, and 1 in 100 are 93.1%, 98.2% and 100%, respectively.

Sample size calculations were performed using PASS v12.0.02.

6. DETERMINATION OF PROTOCOL DEVIATIONS

Major protocol deviations (PDs) (major deviations, see section 6.2) will be listed, even if they are assessed not to influence any of the immunogenicity or safety results for subjects who have been entered into the study and assigned a subject number.

The following criteria will exclude a subject from the Full Analysis Set(FAS). These will be listed in the deviation listing:

- Did not provide both pre- and post-vaccination blood samples
- Laboratory-confirmed influenza infection prior to collection of the day 22 serology sample

Note: Other reasons for deviation may be added to this list, but will be done so prior to unblinding of the study. The reasons highlighted will be used in defining the FAS and Per-Protocol Set (PPS).

6.1 Definition of Protocol Deviations

Major PDs are defined in accordance with International Conference on Harmonization (ICH) E3 as important PDs related to study inclusion or exclusion criteria, conduct of the trial, subject management or subject assessment resulting in the potential to jeopardize the safety or rights of the trial subjects or the scientific value of the trial. PDs will be classified as major and minor.

All PDs will be evaluated before unblinding and most will be classified into the following categories:

- Subject randomized and did not satisfy entry criteria
- Subject received the wrong treatment or incorrect dose
- Subject took an excluded concomitant medication
- Key study procedures missed or performed out of window

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

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

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

Prematurely terminating study participation for reasons such as withdrawal of consent or occurrence of AEs (including death) is not considered as a PD. Any missing assessments that

should have otherwise been collected for that subject later in the study is also not considered as a PD.

6.2 Determination of Protocol Deviations

Deviations from the protocol will be documented on an ongoing basis by the study monitors and lead clinical research associate or designee throughout the study period.

At the time of database lock, prior to unblinding and while the major PDs are being reviewed, the project manager or designee will forward all relevant documentation highlighting PDs to the study statistician. These deviations will be included in the PD document for agreement and will be listed with the PDs in the clinical study report (CSR).

Study PDs can be defined as follows:

- Major PDs arise when subjects who did not meet the Inclusion Criteria, or who met an Exclusion Criterion are entered into the study.

  Other Major PDs may include:
  - Failure to return diary or diary is not completed at all and solicited AE reporting at 30 minutes post vaccination is done.
    - Action to be taken for analysis: Included in the overall Solicited Safety SET, but excluded from the solicited AEs ANALYSIS for the specific time period that reports on Day 1 (6hrs) to Day 7 and sub-periods 6 hrs to Day 3; Day 4 to Day 7.
  - Solicited AE reporting at 30 minutes post vaccination (all data points) not done and diary card is completed.
    - Action to be taken for analysis: Included in the overall Solicited Safety SET, but excluded from the solicited AEs ANALYSIS for the specific time period that reports on 30 minutes assessment only.
  - Failure to return diary or diary is not completed at all and solicited AE reporting at 30 minutes post vaccination is not done.
    - Action to be taken for analysis: Excluded from the overall Solicited Safety SET.
  - Failure to obtain and/or properly document informed consent
  - Informed consent obtained after the initiation of study procedures
  - Use of an unapproved consent form
  - Omitting study procedures required by approved protocol (including not providing pre- and post-vaccination blood samples)
  - Performing a study procedure that is not outlined in the IRB-approved protocol
  - Subject failure to return diary card within 7 days of scheduled return period
  - Subject failure to return diary card
  - Failure to report an SAE/AESI
  - Drug dispensing / dosing error (this includes administration of a vaccine that has been subject to a temperature deviation, without pre-vaccination approval for the product to be administered)

  - Failure to securely control the study product
  - Receive prohibited medication (to be medically reviewed after database lock for confirmation, see section 3.4)
  - Study visit outside of window, if it affects the safety or welfare of the research participants or others, the rights of participants or others, or the integrity of the study design
- Minor PDs arise when subjects who have been entered in the study deviate from the approved Study Protocol.

  Minor PDs may include:
  - Study visit is not attended within the time window specified by the Study Protocol.
  - Study procedure conducted out of timeframe
  - Copy of consent form not given to subject during the informed consent process
  - Missing original signed consent, but have a copy of the subject signed consent

PD listings will be reviewed by Seqirus prior to the finalisation of the population datasets, which will occur prior to unblinding. The list will be used to determine which subjects should be excluded from either the FAS or the PPS.

6.3 Exclusions of Individual Values for Safety Analysis

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

Table 6-1: Implausible Solicited Adverse Events

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

7. ANALYSIS SETS

There will be five analysis sets defined for the study analyses.

7.1 All Enrolled Set

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

Demography and baseline characteristics tables will be produced on the All Enrolled Set.

7.2 Exposed Set

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

7.3 Full Analysis Set (FAS) Immunogenicity

All subjects in the All Enrolled Set who are randomized, receive at least one study vaccination and provide immunogenicity data at Day 1 and Day 22.

The FAS will be used to produce summaries and listings of subject characteristics.

In case of vaccination error, subjects in the FAS Immunogenicity will be analyzed "as randomized" (i.e., according to the vaccine a subject was designated to receive, which may be different from the vaccine the subject actually received).
See section 4.1 for details on how to handle subjects randomized in the wrong stratum.
If a subject is unblinded during the study, he/she will be included in the FAS Immunogenicity.

7.4 Per Protocol Set (PPS) Immunogenicity

The Per-Protocol Set (PPS) will comprise all subjects in the FAS Immunogenicity who do not have any PDs that are medically assessed as potentially impacting on immunogenicity results.

The PPS will be the primary set of interest for the primary/secondary immunogenicity analysis and a supporting analysis will be performed using the FAS Immunogenicity. Membership of the PPS will be determined prior to unblinding the study.

Duplicate tables of primary and secondary immunogenicity analyses may also be produced based on the FAS Immunogenicity if there is >1% difference in the total number of subjects between the PPS and the FAS Immunogenicity. The decision to produce tables based on the FAS Immunogenicity will be made by Seqirus after analysis sets are finalised and prior to unblinding.

Examples for subjects excluded due to other reasons than PDs are subjects who withdrew informed consent, and subjects with RT-PCR-confirmed ILI prior to Day 22, as documented by the central laboratory.

In case of misrandomization with regard to treatment arm, the subject is excluded from the PPS.

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

7.5 Safety Set

The Safety Set will comprise all subjects in the Exposed Set who received at least one dose or partial dose of Study Vaccine and have provided any evaluable follow-up safety data. The safety set will be used to produce summaries and listings of all safety data.

Solicited Safety Set

All subjects in the Exposed Set with any solicited AE data.

Unsolicited Safety Set

All subjects in the Exposed Set with unsolicited AE data.

Overall Safety Set

All subjects who are in the solicited safety set or in the unsolicited safety set.
In case of vaccination error, subjects will be analyzed as "treated" (i.e., according to the vaccine a subject receives, rather than the vaccine to which the subject is randomized).

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

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

8. GENERAL ISSUES FOR STATISTICAL ANALYSES

8.1 Adjustment for Covariates

The complete set of covariates that may be used in the model to calculate the adjusted GMT ratio may include treatment group (3 treatments), age sub-group (3 categories, (≥65-74, ≥75-84, and ≥85years), sex (male or female), influenza vaccination received prior year (Y or N), pre-vaccination mean GMT titer (value) and investigator site (site identifier)

8.2 Handling of Dropouts, Missing Data

Missing, unused and spurious data will be dealt with as such. There is no intention to implement any procedure for replacing missing data.

Titer values recorded as <10 will be recorded as 5.

8.2.1 Safety Data

For unsolicited AEs, the entire study period will be divided into the following Intervals: Day 1 – Day 22; Day 23 – Day 181; and Day 1 – Day 181 by maximal severity and by vaccine group.

For solicited AEs, the solicited study period (30 min post-vaccination on Day 1 through Day 7 will be divided into: Day 1-3, Day 4-7, Day 1-7 by maximal severity and by vaccine group, excluding the 30-minute measurement on Day 1, which will be summarized separately.

No imputation of missing solicited or unsolicited AEs will be used. The percentage of subjects with missing solicited AE assessments (e.g. missing Patient Diary) and missing Safety Phone Calls or Safety Assessments will be reported for each time period.

8.2.2 Immunogenicity Data

Missing immunogenicity values are considered missing completely at random (MCAR) and therefore will not contain information that impact the result of the analysis (i.e., not informative). Imputation methods will therefore not be used.

The PPS will be used for the primary immunogenicity analysis and a supporting analysis will be performed using the FAS Immunogenicity, as noted in **Section 7.3 FAS Immunogenicity**. Duplicate tables of primary and secondary immunogenicity analyses may also be produced based on the **FAS Immunogenicity** if there is >1% difference in the total number of subjects between the PPS and the **FAS Immunogenicity**.

8.2.3 Efficacy Data

NA

8.3 Multicenter Studies

There will be no adjustment for multiple centers.

8.4 Multiple Comparisons and Multiplicity

No adjustment for multiple comparison and multiplicity will be used.

8.5 Immunogenicity/Safety/Other Subsets

Not applicable. Immunogenicity and safety data are collected from all patients.

8.6 Subgroups

Additional subgroup analyses will be conducted for both safety and immunogenicity assessments. Immunogenicity endpoints will also be stratified by the for the following subgroups:

- Age at enrollment (≥65-74, ≥75-84, and ≥85 years)
- Sex
- Race
- Previous influenza vaccination in the past 5 years (yes/no)
- Comorbidity/risk (yes/no, defined as assessment score < 50 or ≥50 based on scale described in Section 5.1.2 of the protocol)

Safety analysis of any unsolicited AEs and of any local, any systemic, or any other solicited AEs (all AEs combined for each such category) will be performed by stratifying for the following subgroups:

- Age at enrollment (≥65-74, ≥75-84, and ≥85years)
- Sex
- Race

8.7 Data Transformation

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

8.8 Derived and Computed Variables

Demographics

Age and Body Mass Index will be calculated according the following formulas:

Age:

Round [(Date of Visit 1 – Date of Birth + 1) / 365.25]

Body Mass Index (kg/m^2):

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

Immunogenicity

Values below the limit of quantification (LQ = 10) recorded as "< LQ" and values lower than the limit of quantification have been set to half that limit (5).

The rate of seroconversion is defined as the percentage of subjects with either a prevaccination HI titer < 1:10 and a post vaccination HI titer ≥ 1: 40 or a prevaccination HI titer ≥ 1:10 and a ≥ 4-fold increase in post vaccination HI titer.

Seroconversion is defined as binary variable for subjects with non-missing values pre-vaccination and post-vaccination as:

= 1, if seroconverted (defined as the percentage of subjects with either a pre-vaccination HI titer < 1:10 and a post-vaccination HI titer ≥ 1:40 or a pre-vaccination HI titer ≥ 1:10 and a minimum 4-fold rise in post-vaccination HI antibody titer)

= 0, otherwise

Geometric Mean Titer (GMT)

Serum HI antibody levels of all participants will be determined in triplicate (HI1, HI2 and HI3) on serum separated from the whole blood. Pre- and post-vaccination samples will be titrated in duplicate, simultaneously. The titer assigned to each sample shall be the geometric mean of three independent determinations i.e. Assigned titer=exp[(log(HI1) +log(HI2) +log(HI3))/3].

GMT will be based on the following:

- HI antibody titer for each strain: All analyses involving HI antibody titer (namely group GMT within a treatment group) will be performed on the log scale and the resultant summary statistic back-transformed to derived GMT.

The GMT will be calculated using the following formula:

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

where $t_1, t_2, \ldots, t_n$ are n observed immunogenicity titers. The 95% confidence intervals for GMT will be calculated as $10^{\{M - t_{0.975,n-1}SE)\}}$, $10^{\{M + t_{0.975,n-1}SE)\}}$; where M and SE are the means and standard error of logarithm base 10 -transformed titers, respectively.

Geometric Mean Ratio (GMR)

GMRs measure the changes in immunogenicity titers *within* subjects.

The GMR will be calculated using the following formula:

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

where, for n subjects, t_{ij} and t_{ik} are observed immunogenicity titers for subject i at time-points j and k, $j \neq k$. The 95% confidence intervals for GMR will be calculated as $10^{\{M - t_{0.975,n-1}SE)\}}$, $10^{\{M + t_{0.975,n-1}SE)\}}$; where M and SE are the means and standard error of $\log_{10}(t_{ij}) - \log_{10}(t_{ik})$ respectively.

Duration in the Study

Duration in the study is defined in days as:

[Last visit date (Day 181)[a] – Enrollment date (Day 1) + 1]

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

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

Solicited AEs

For details see section 13.2.

Unsolicited AEs

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

- **Non-Treatment Emergent**: start date before the date of injection of study vaccine.

- **Treatment Emergent**: start date on or after the date of injection of study vaccine or, AE increase in severity, including to "serious" AE.

If start date is equal to the first date of injection, then "timing" variable ("Did event start before or after vaccination?") will be used to define whether the AE occur before or after the injection. If an AE happened on the same day of injection and the time stamp is missing, then the AE is assumed to be treatment emergent.

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

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

- If the partial end date is before (<) the vaccination (i.e., year or year & month is/are before the study vaccination year or year & month) then the AE is not treatment-emergent before vaccination phase.
- If the partial start date is equal or after (≥) the first study vaccination (i.e., year or year & month is/are after or the same as the first study injection year or year & month) then the AE is considered treatment-emergent.

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

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

Vaccination-related AEs are those for which the cause has been evaluated by the investigator, and recorded as related.

Previous and Concomitant Medications

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

All other medications are **concomitant**.

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

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

8.9 Analysis Software

All analyses will be performed using SAS Software version 9.3 or higher.

9. STUDY SUBJECTS

9.1 Disposition of Subjects and Withdrawals

The number of subjects screened, enrolled into the study, in each study analysis set, who completed the study, and the reasons for any premature discontinuation from the study will be presented in summary tables by treatment group, by age cohort (≥65-74, ≥75-84, and ≥85years) and overall.

The number of subjects who are excluded from each of the FAS Immunogenicity and PPS will be summarized by treatment group, by age cohort (≥65-74, ≥75-84, and ≥85years) and overall.

10. DEMOGRAPHICS AND OTHER BASELINE CHARACTERISTICS

10.1 Demography

Descriptive statistics (mean, SD, median, minimum and maximum) for age, height, weight, BMI, and comorbidity score at enrollment will be calculated overall and by vaccine groups.

Distributions of subjects by sex, ethnic origin (race, ethnicity), and previous vaccination status will be summarized overall, and by vaccine group.

Demographic data will be tabulated for the FAS Immunogenicity, PPS and the All Enrolled analysis sets.

10.2 Medical History

The numbers and percentages of subjects with medical history will be presented by Medical Dictionary for Regulatory Activities (MedDRA) system organ class (SOC) and preferred term (PT) by vaccine group and overall. Medical history data will be tabulated for the All Enrolled Set.

10.3 Concomitant Medications

Use of concomitant medication will be presented by treatment, therapeutic area, and preferred drug name.

Concomitant medications are all medications taken during the study period, including those started before but ongoing at vaccination.

If a start date for a medication is partially or fully missing, and it is unclear as to whether the medication is prior or concomitant, it will be assumed that it is concomitant.

Medications will be coded using the WHO Drug dictionary.

11. PRIMARY AND SECONDARY ENDPOINTS ANALYSIS

11.1 Analysis of Co-Primary Immunogenicity Objectives

11.1.1 Co-PRIMARY ENDPOINTS

The immunogenicity of study vaccines will be assessed 21 days (i.e, on Day 22) after vaccine administration by measuring the HI antibody titers to the four virus strains included in the vaccines.

The non-inferiority of aQIV compared to aTIV-1, and to aTIV-2 will be assessed for the eight co-primary endpoints of HI geometric mean titer (GMT) and SCR for each virus strain included in the vaccines as follows:

- The GMT ratio* for the A/H1N1 strain;
- The GMT ratio for the A/H3N2 strain;
- The GMT ratio for the B strain (Yamagata lineage);
- The GMT ratio for the B strain (Victoria lineage);
- The difference between the SCR**for the A/H1N1 strain;
- The difference between the SCR for the A/H3N2 strain;
- The difference between the SCR for the B strain (Yamagata lineage);
- The difference between the SCR for the B strain (Victoria lineage).

Immunogenicity results obtained from aTIV-1 and aTIV-2 for both A/H1N1 and A/H3N2 strains will be pooled for comparison with aQIV.

**The GMT ratio is defined as the geometric mean of the post-vaccination (Day 22) HI titer for aTIV-1 (or aTIV-2) over the geometric mean of post-vaccination (Day 22) HI titer for aQIV.*
***The SCR is defined as the percentage of subjects with either a pre-vaccination HI titer < 1:10 and a post-vaccination HI titer ≥ 1: 40 or a pre-vaccination HI titer ≥ 1:10 and a ≥ 4-fold increase in post-vaccination HI titer.*

11.1.2 Statistical Hypotheses

Noninferiority of aQIV to aTIV-1 and aTIV-2

To demonstrate that vaccination with aQIV elicits an immune response that is not inferior to that of an aTIV containing the same virus strains as the US licensed adjuvanted influenza vaccine (Fluad, aTIV-1), and an aTIV containing the alternate B strain (aTIV-2) among adults ≥65 years of age. according to the FDA Guidance on seasonal inactivated influenza vaccines *(Guidance for Industry Clinical Data Needed to Support Licensure of Seasonal Inactivated Influenza Vaccines 2007)*.

aQIV will be considered to be non-inferior to aTIV if, for each of the four strains, the following statistical criteria are met:

- The upper bound of the two-sided 95% CI for the ratio of the GMTs does not exceed 1.5. The GMT ratio will be calculated by GMT_{aTIV}/ GMT_{aQIV}
- The upper bound of the two-sided 95% CI for the difference between the SCRs does not exceed 10%. The difference in SCR will be calculated by $SCR_{aTIV} - SCR_{aQIV}$

In mathematical notation, the statistical hypotheses to be tested for the primary immunogenicity analysis correspond to:

H0: $GMTri > 1.5$, for any strain

Ha: $GMTri \leq 1.5$, for all strain

and

H0: $Di > 10$, for any strain

Ha: $Di \leq 10$, for all strain

where *GMTri* is any of the 4 strain-specific post immunogenicity dose GMT ratios namely,

- $GMT_{aTIV-1/} GMT_{aQIV}$ for B/Yamagata strain
- $GMT_{aTIV-2/} GMT_{aQIV}$ for B/Victoria strain
- Pooled $GMT_{(aTIV-1}$ and $_{aTIV-2)} /GMT_{aQIV}$ for A/H1N1 strain
- Pooled $GMT_{(aTIV-1}$ and $_{aTIV-2)} /GMT_{aQIV}$ for A/H3N2 strain

and *Di* is the 4 strain-specific post dose SCRs (π_{aTIV-1}, $\pi_{aTIV-2,}$ π_{aQIV}) difference, namely,

- $\pi_{aTIV-1} - \pi_{aQIV}$ for B/Yamagata strain
- $\pi_{aTIV-2} - \pi_{aQIV}$ for B/Victoria strain
- Pooled $\pi_{(aTIV-1}$ and $_{aTIV-2)}$-π_{aQIV} for A/H1N1 strain
- Pooled $\pi_{(aTIV-1}$ and $_{aTIV-2)}$-π_{aQIV} for A/H3N2 strain

where π_v denotes the SCRs for the *v*th vaccine (aQIV, aTIV-1, aTIV-2).

CBER Immunogenicity Criteria

The endpoints of percent of subjects achieving SC and HI titer ≥ 1:40 at Day 22 will be assessed against the criteria described in CBER Guidance Clinical Data Needed to Support the Licensure of Seasonal Inactivated Influenza Vaccines (FDA, 2007):

- The lower bound of the two-sided exact 95% CI for the percentage of subjects achieving SC for HI antibody should meet or exceed 30% and
- The lower bound of the two-sided exact 95% CI for the percentage of subjects achieving an HI antibody titer ≥1:40 should meet or exceed 60%

Assuming that $Y_{jk} \sim B(1, \pi_k)$, $Z_{jk} \sim B(1, \tau_k)$, j=1,..,n, identical and independent Bernoulli distributed random variables with π_k representing the unknown SC proportion postvaccination

and τ_k representing the unknown SP (HI titres $\geq$ 1:40) proportion postvaccination in aQIV for strain k, where k denotes the 4 strains contained in aQIV vaccines, CBER requirements for the aQIV vaccine group translate into following hypothesis

$H_{0k}^{(SC)}: \pi_k \leq 0.3$ vs $H_{1k}^{(SC)}: \pi_k > 0.3$

$H_{0k}^{(SP)}: \tau_k \leq 0.6$ vs $H_{1k}^{(SP)}: \tau_k > 0.6$

All hypotheses related to CBER criteria will be tested on unadjusted 5% significance levels.

11.1.3 Analysis Sets

The PPS will be used for the primary immunogenicity analysis and a supporting analysis will be performed using the FAS Immunogenicity. Duplicate tables of primary and secondary immunogenicity analyses may also be produced based on the FAS if there is >1% difference in the total number of subjects between the PPS and the FAS .

11.1.4 Statistical Methods

All statistical analyses for HI titers will be performed on the logarithmically transformed (base 10) values. Individual HI titers below the detection limit (<10) will be set to half of that limit (5).

Co-primary immunogenicity endpoints of GMT and SCR for each virus strain contained in the vaccine will be assessed for subjects ≥ 65 years overall. For A/H1N1 and A/H3N2 strains the two aTIV treatment groups will be pooled.

Primary analysis will be performed for subjects ≥ 65 years using the PPS. The difference in SCRs will be presented with exact 95% (CIs). Miettinen and Nurminen method may be used if convergence issues (Miettinen OS, Nurminen M., 1985). Each of the four strains will be analyzed separately.

To determine the GMT ratio (adjusted analysis) a general linear model (GLM) will be fitted on log transformed (base ten) post-vaccination HI titer as the outcome variable and terms for covariates: vaccine treatment, pre-vaccination HI titer, age stratum, sex, vaccination history, age-by-vaccine interaction and study site. Potential covariate interaction effects will also be examined in the fit of the GLM. From the model, an adjusted difference in least square means (on the log scale) will be produced with 95% confidence limits. The estimated difference and the confidence limits will be back-transformed to obtain an *adjusted GMT ratio* with 95% confidence limits. Each of the four strains will be analyzed separately. The adjusted GMT ratio will be the result for which the non-inferiority assessment of the HI GMT co-primary endpoint will be based on.

The statistical models might be reduced in case they fail to converge.

The complete set of covariates that may be used in the model to calculate the adjusted GMT ratio will include treatment group (3 treatments), age sub-group (3 categories, (≥65-74, ≥75-84, and ≥85years), sex (male or female), influenza vaccination received prior year (Y or N), pre vaccination mean GMT titer (value) and investigator site (site identifier)

The GLM specification (for each strain) is:

- *Adjusted Analysis GMT Model:* Log10-transformed Post-vaccination HI Titer = Vaccine + Age Strata + Gender + Vaccination History [y/n] + Log10-transformed Pre-vaccination HI Titer + Site + Age Strata*Vaccine.

For any strain, the interaction term *Age Strata*Vaccine* will be removed from the fit of the model if it is assessed to be not significant at the 0.05 level.

The measure of the *unadjusted* GMT ratio (and CI's) based on post-vaccination GMTs only will also be presented.

In line with the FDA Guidance on seasonal inactivated influenza vaccines *(Guidance for Industry Clinical Data Needed to Support Licensure of Seasonal Inactivated Influenza Vaccines 2007)*. aQIV will be considered to be noninferior to aTIV if, for each of the four strains, the following statistical criteria are met:

- The upper bound of the two-sided 95% Confidence Interval (CI) for the ratio of the GMTs does not exceed 1.5. The GMT ratio will be calculated by GMT_{aTIV}/ GMT_{aQIV}
- The upper bound of the two-sided 95% CI for the difference between the SCRs does not exceed 10%. The difference in SCR will be calculated by $\pi_{aTIV} - \pi_{aQIV}$

To achieve the CBER criteria:

- The lower bound of the two-sided 95% confidence interval for the percentage of subjects achieving SC for HI antibody should meet or exceed 30%
- The lower bound of the two-sided 95% confidence interval for the percentage of subjects achieving an HI antibody titer ≥ 1:40 should meet or exceed 60%

Binary data (ie, percentages of subjects with SC and with titer ≥ 1:40) will be summarized for each group using unadjusted estimates and will be reported together with two-sided exact 95% CIs. No multiplicity adjustment to the CI levels will be implemented.

Handling of missing values for Immunogenicity Data:

For immunogenicity data, it may be reasonable to consider missing immunogenicity values as missing completely at random (MCAR), ie, not informative. Therefore, the key secondary analysis will comprise a complete case analysis only, without introducing any bias. Imputation methods will not be used.

11.2 Analysis of Secondary Objectives

11.2.1 Analysis of Secondary Immunogenicity Objective(s)

Characterization of immunogenicity of aQIV, aTIV-1 and aTIV-2:

The measures of immunogenicity of aQIV, aTIV-1 and aTIV-2 will be assessed in terms of HI antibodies. Serum HI antibody titers against the 4 influenza vaccine strains will be used to calculate:

- GMT: Geometric mean of HI titers on Day 1 and Day 22

- GMR: The geometric mean of the fold increase of post-vaccination HI titer over the pre-vaccination HI titer (Day 22/Day 1)
- The percentage of subjects with a titer ≥ 1:40 at Day 1 and Day 22
- SCR: the percentage of subjects with either a pre-vaccination HI titer < 1:10 and a post-vaccination HI titer ≥ 1:40 or a pre-vaccination titer ≥ 1:10 and a ≥ 4-fold increase in post-vaccination titer on Day 22

Superiority of immunologic response for aQIV vs aTIV:

Superiority of aQIV over aTIV-1 and aTIV-2 for antibody response to the alternate B strain will be assessed using the adjusted GMT ratio (GMT_{aTIV}/GMT_{aQIV}) and difference in SCR (SCR_{aTIV} - SCR_{aQIV}) at Day 22. Point estimates and 95% confidence limits will be obtained as described for the primary endpoint. Superiority will be declared if the upper limit of the two-sided 95% CI for the difference in ($SCR_{aTIV} - SCR_{aQIV}$) is < 0 and the upper limit of the two-sided 95% CI for the GMT ratio (GMT_{aTIV}/GMT_{aQIV}) is < 1 for both B strains.

The same GLM as was used for the primary analysis will be used to determine the adjusted GMT ratio for assessing superiority.

11.2.2 Statistical Hypotheses

No statistical testing will be performed for the first secondary immunogenicity objectives.

Superiority of B-strain response (GMTs and proportions of subjects with SC):

For superiority of aQIV B strain response, the hypotheses are that immunologically aQIV is superior compared to aTIV-1 and aTIV-2 for the B strain that is not included in each TIV vaccine separately. In mathematical form, these are:

$$H_{0k}^{(GMT)}: GMTr_k \geq 1 \text{ vs } H_{1k}^{(GMT)}\ GMTr_k < 1 \quad \text{for } k=1,2$$

and

$$H_{0k}^{(SCR)}: D_k \geq 0 \text{ vs } H_{1k}^{(SCR)}\ D_k < 0 \quad \text{for } k=1,2$$

where $GMTr_k$ is the adjusted ratio of geometric mean titer $GMT_{aTIV\text{-}k}/GMT_{aQIV}$ of mismatched B strain for k=1, 2, and D_k is the differences $\pi_{aTIV\text{-}k} - \pi_{aQIV}$ of mismatched B strain Day 22 SCR differences for k=1,2.

11.2.3 Analysis Sets

The secondary objective superiority testing will be performed using the PPS.

11.2.4 Statistical Methods

All statistical analyses for HI titers will be performed on the logarithmically transformed (base 10) values. Individual HI titers below detection limit (<10) will be set to half of that limit (5).

Unadjusted GMTs, GMRs and pertaining two-sided 95% CIs will be calculated assuming log-normal distribution of the titers and will be completed by providing minimum, maximum and median titers for each vaccine group.

Binary data (ie, percentages of subjects with SC and with titer ≥ 1:40) will be summarized for each group using crude estimates and will be reported together with 2-sided exact 95% CIs. No multiplicity adjustment to the CI levels will be implemented.

For immunogenicity data, it may be reasonable to consider missing immunogenicity values as missing completely at random (MCAR), i.e., not informative. Therefore, the key secondary analysis will comprise a complete case analysis only, without introducing any bias. Imputation methods will not be used.

The following figures will be also produced:

- GMT Reverse Cumulative Distribution Curve – Overall by Strain
- GMT Reverse Cumulative Distribution Curve – by Age Cohort
- GMT Reverse Cumulative Distribution Curve – by Study Treatment

11.2.5 Analysis of Secondary Safety Objective(s)

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

- Vaccine exposure.
- Solicited local and systemic AEs.
- Unsolicited AEs.
- SAEs, AE leading to withdrawal, NOCD, AESI

11.2.5.1 Analysis of Extent of Exposure

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

11.2.5.2 Solicited Adverse Events

The safety completeness analysis on solicited AEs aims to identify subjects who completed diary cards, irrespective of severity. The analysis will show the number of subjects with valid data by solicited AE and time point. Valid data in the context of the safety completeness analysis are all data entered in the diary card (including implausible values) except “Not done/unknown”.

Four summaries will be produced:

1. The frequencies of subjects who provide diary cards by vaccine group.

2. For each solicited AE, the frequencies of subjects with valid data will be presented by vaccine group and timepoint: 30 min, 6h –Day 3, Day 4-7, 6h - Day 7, Day 1 – 7 (with/without 30 mins).
3. For each type of solicited AE (local, systemic) and indicators of solicited AEs, such as analgesic use, the frequencies of subjects with valid data by vaccine group, aggregated over time points: 30 min, 6h –Day 3, Day 4-7, 6h - Day 7, Day 1 – 7 (with/without 30 mins).
4. For each solicited AE, the frequencies of subjects with valid data by vaccine group, aggregated over time points: 30 min, 6h –Day 3, Day 4-7, 6h - Day 7, Day 1 – 7 (with/without 30 mins).
5. For the corresponding percentages, the denominator will be the respective numbers of exposed subjects, i.e., subjects who received a vaccination and were still in-study for that time point or time interval, irrespective of whether a diary card was present or not.

All analyses will be based on the 'as treated' Solicited Safety Set.

11.2.5.3 Solicited Local and Systemic AEs

For details please refer to section 7.1.1 of the protocol.

Only solicited local and systemic AEs reported in the diary card will be analyzed. Implausible measurements will not be taken into consideration in the analysis (see section 6.3).

Solicited AEs will be reported at 30 minutes, at 6 hours on Day 1 and then daily until Day 7 using structured diaries. The analyses of solicited AEs will be done separately for 30 minutes and based on three intervals: 6h - Day 3, Day 4 - 7 and 6h - Day 7, each without 30 minutes' data. In addition, solicited AEs ongoing after Day 7 will be presented as unsolicited AE.

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

Type I: none (0 mm), any (1-24 mm, 25-50 mm, 51-100 mm, >100 mm)

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

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

- Brighton:
  - <38.0,
  - 38.0 - 38.4
  - 38.5 - 38.9
  - 39.0 - 39.4
  - 39.5 - 39.9
  - 40.0 – 40.4
  - 40.5 – 40.9

- ≥41.0°C

- CBER:

  - 38.0–38.4

  -38.5-38.9

  -39.0-40.0

  >40°C

- <38.0, ≥38.0 °C

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

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

1. Daily reports of subjects with solicited AEs.
2. Time of first onset of solicited AEs 30 min measurement (after 30 minutes).
3. Solicited AEs, maximum event severity by event and interval 6h - Day 3, Day 4 -7, and 6h - Day 7, each without 30 min.
4. Duration of solicited AEs, including ongoing AE after Day 7.
5. Solicited AEs and indicators of solicited AEs, occurrence of at least one event by category (local, systemic) and interval 6h-Day 3, Day 4-7 and 6h-Day 7, each without 30 min.

For each of the time points or time intervals presented in the summaries, only subjects with at least one plausible observation (i.e., any non-missing values but excluding "Not done/unknown" and implausible values) for the solicited AEs in the interval of interest will be considered. Subjects without plausible data (i.e. missing values or reported as "Not done/unknown" and implausible values) will be removed from the denominator to prevent a downward bias (towards zero).

Level 1: Daily reports of solicited AE

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

Level 2: Time of first onset of solicited AEs

The **time of first onset** is defined, for each subject, for each solicited AE, as the time point at which the respective solicited AE first occurred. For erythema, induration, and ecchymosis the

following threshold will be used: ≥ 1 mm. The summary will provide the frequencies and percentages of subjects with first onset of each solicited AEs by vaccine group and by each time point.

The following example is used to illustrate how the onset data is selected:

Suppose four subjects, who receive vaccination A, have the following post-vaccination data for solicited AE XY

Table 11-1: Example for Time to First Onset of Solicited Adverse Events

Vaccination	Subject Number	6 Hours	Day 2	Day 3	Day 4	…	Day 7
1	001	None	Severe	Moderate	None	…	None
	002	Mild	None	None	Moderate	…	Missing
	003	Moderate	Mild	None	Severe	…	Mild
	004	Mild	Mild	None	None	…	Not done

Level 3: Solicited AEs, maximum event severity by event and interval

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

Level 4: Number of days with solicited AEs

The number of days with the AE is defined irrespective of severity. This means at least 'mild' solicited AE that are assessed qualitatively and ≥ 1 mm for erythema and induration and ecchymosis. If a solicited AE continues beyond Day 7 the date of resolution is recorded and the entirety of the duration is included in the calculation. The following example is used to illustrate how the duration is calculated:

Suppose six (6) subjects, who received a vaccination have the post-vaccination solicited AE data shown in the table below. In addition, there are unsolicited AE reports indicating that the AE in subject 003 and 006 continued until Day 12 and Day 8, respectively. For subject 003 the number of days is calculated as 6+5 and for subject 006 as 3+1. Missing values ('Missing') are not taken into consideration

Table 11-2: Example for Number of Days with Solicited Adverse Events

Subject Number	6 Hours	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	No. of days
001	None	Severe	Moderate	None	None	None	None	2

Subject Number	6 Hours	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	No. of days
002	Mild	None	None	Moderate	Moderate	Moderate	Missing	4
003	Moderate	Mild	None	Severe	Severe	Severe	Mild[a]	11
004	None	None	None	None	None	None	Not done	0
005	None	Mild	Mild	Missing	Missing	Missing	Missing	2
006	Severe	None	Mild	None	None	None	Severe[b]	4

[a] continued until Day 12; [b] continued until Day 8

The frequency distribution of the number of days will be provided in a summary table by vaccine and by AE.

Level 5: Solicited AEs, occurrence of at least one event by category (local, systemic) and interval.

The **occurrence of at least one solicited AE** is defined as "any" for a subject if he/she reports greater than "none", ≥ 1 mm for erythema, ecchymosis and induration) for the respective event and "none" otherwise. The occurrence of at least one solicited AE (i.e., none versus any) will be summarized by category (i.e., local, systemic, any), by vaccine group, by vaccination (after each vaccination and after any vaccination) and by time interval.

Medications to treat or prevent pain or fever will be summarized by frequencies and percentages of subjects reporting use of the medications by interval 30 min, 6h - Day 3, Day 4 -7, and 6h - Day 7.

11.2.5.4 Analysis of Unsolicited Adverse Events

If the reporting source is collected in the study, specify the following: The first-line analysis will use unsolicited AE data from all reporting sources combined. A second-line analysis will encompass the analysis of unsolicited AEs by source (medical records, study specific worksheet).

All the unsolicited AEs occurring during the study, judged either as probably related, possibly related, or not related to vaccination by the investigator, will be recorded through the day 22 clinic visit. AEs leading to withdrawal, AESI, NOCD and SAEs up to Day 181 will be captured. The original verbatim terms used by investigators to identify AEs in the CRFs will be mapped to lowest level term using the MedDRA dictionary. The unsolicited AEs will then be grouped by MedDRA preferred terms into frequency tables according to primary system organ class. AEs judged by the investigator as at least possibly related to study vaccine will be summarized by vaccine group, according to system organ class and preferred term within system organ class. When an unsolicited AE occurs more than once for a subject, the maximal severity and strongest relationship to the vaccine group will be counted.

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

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

- Onset between Day 1 and Day 22.
- Onset between Day 23 and Day 181.
- Onset between Day 1 and Day 181

The analysis of unsolicited AEs comprises the following categories:

- Any unsolicited AE.
- Possibly or probably related unsolicited AEs.
- Unsolicited AEs leading to death.
- Serious adverse events (SAEs).
- Possibly or probably related SAEs.
- Unsolicited AEs leading to premature withdrawal from study.
- Unsolicited AEs leading to NOCD.
- Unsolicited AEs of special interest.

Solicited AEs continuing beyond Day 7 will be coded by MedDRA and will also be reported as unsolicited AEs.

11.2.5.5 Combined Solicited and Unsolicited Adverse Events

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

11.2.5.6 Concomitant Medication

The numbers and percentages of subjects reporting concomitant medications will be tabulated overall and by vaccine group and study period (Treatment by Day 1-21, Day 22-Day 181). Medications (generic drug name) will be coded using the World Health Organization (WHO) Drug dictionary (see section 8.7 for definition).

11.3 Analysis of Exploratory Objectives

The exploratory objectives are:

1. Additional optional objectives include characterization of the immunogenicity of aQIV using other immunological assays (e.g., virus neutralization [MN] or anti-neuraminidase antibody assays may be performed)
2. To explore the association between immune response after administration of aQIV or the aTIV-1, containing the same virus strains as the US-licensed adjuvanted trivalent influenza vaccine, or the aTIV-2 containing the alternate B strain by baseline characteristics.

Exploratory immunogenicity objective 1

Exploratory objectives related to the MN-assay, immune response to heterologous influenza strains, or different antigens will be evaluated using similar methods for log normal, binomial data, and GMT and SCR as described in the statistical method for primary and secondary immunogenicity analysis.

Exploratory immunogenicity objective 2

- GMTs: Geometric mean of HI titers prevaccination (Day 1) and at Study Exit Visit
- Percentage of subjects with four-fold rise

Analyses of the exploratory immunogenicity endpoints will be performed with adjustment for covariates including prevaccination titer, vaccination history, age and gender to evaluate the contribution of these factors to variations in the immune response. The covariate adjustment will be performed with all of the specified covariates in the general linear model.

The full model specification for the GMT titers (for each strain) will be:

Log-transformed Post-Vaccination HI Titer = Vaccine + Age Strata + Gender+ Vaccination History + Log-transformed Prevaccination HI Titer.

The full model specification for the SCR rates will be:

SCR = Vaccine + Age Strata + Gender + Vaccination History + Log-transformed Pre-vaccination HI titer

SCRs will be analyzed using a logistic regression model, and GMT titers will be log transformed (base 10) and analyzed with a general linear model. Pre-vaccination titers will be log transformed (base 10) and entered as a continuous covariate. Vaccination history will be a categorical variable indicating whether or not an individual received an influenza vaccination in the previous year. If the proportion vaccinated in the previous year is close to 100% then this covariate may be omitted to avoid creating an unstable model. Age will be entered according to the predefined strata (≥65-74, ≥75-84, and ≥85years). For the logistic regressions, the odds ratios will be presented with 95% CIs for each effect in the model. For the GLM least square mean estimates for the GMT ratio will be presented with 95% confidence limits for each effect in the model.

11.4 Interim Analysis

No interim analysis is planned for this study.

12. EFFICACY ANALYSIS

NA.

13. DATA MONITORING COMMITTEES

An independent Data Monitoring Committee (DMC) will not be utilized for the study.

14. CHANGES TO PLANNED ANALYSES FROM PROTOCOL

All FAS in the protocol (dated 17MAY2017) corresponds to FAS immunogenicity in this document. The protocol is to be updated to match the language in this document.

15. REFERENCES

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16. REVISION HISTORY

The following table outlines amendments to the Final/V 1.0 of the SAP

Version #	Page #	Reason for change	Description of Change
1	All	New document	n/a

Seqirus SIGNATURE PAGE

Prepared by:

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Document Title:	Clinical Study Report: Section 16.1.9. Statistical Analysis Plan (11-Jun-2018)
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Date:	11 FEB 2019
I confirm scanned document is an exact copy having all the same information as the original paper document. I have verified the completeness, correctness and readability of the scanned document with the original paper document.	
Signature:	[redacted]