STATISTICAL ANALYSIS PLAN

Study Title:	A Phase 3 Randomized, Observer-Blind, Multi-center, Controlled Study to Evaluate Safety, Immunogenicity, and Lot-to-Lot Consistency of an Adjuvanted Cell Culture-Derived, H5N1 Subunit Influenza Virus Vaccine in Healthy Adult Subjects ≥18 years of Age
Study Number:	V89_18
Protocol Version and Date:	3.0, 15 MAR 16
Phase of Development:	Phase 3
Sponsor:	Seqirus Inc.
Plan Prepared by:	[redacted]
Version and Date:	Version 3.0: 15 Nov 17
Approvers:	[redacted]

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LIST OF ABBREVIATIONS

AE	Adverse Event
AESI	Adverse Events of Special Interest
ANCOVA	Analysis of Covariance
BARDA	Biomedical Advanced Research and Development Authority
BMI	Body Mass Index
CBER	Center for Biologics Evaluation Research
CHMP	Committee for Medical Products for Human Use
CI	Confidence Interval
CRF	Case Report Form
CRO	Clinical Research Organization
CSR	Clinical Study Report
CST	Clinical Study Team
D	Study Day
DMC	Data Monitoring Committee
EDC	Electronic Data Capture
FAS	Full Analysis Set
GMR	Geometric Mean Ratio
GMT	Geometric Mean Titer
HHS	Department of Health and Human Services
HI	Hemagglutination Inhibition
ICF	Informed Consent Form
IP	Investigational Product
IRT	Interactive Response Technology (for randomization)
LQ	Limit of Quantification
MCAR	Missing completely at Random
MedDRA	Medical Dictionary for Regulatory Activities
N	Number of Subjects

NOCD	New Onset of Chronic Disease
PD	Protocol Deviation
PPS	Per-Protocol Set
RC	Reminder Call
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis System
SC	Safety Call
SD	Standard Deviation
SDA	Source Data Agreement
SP	Statistical Programmer
SUSAR	Suspected Unexpected Serious Adverse Reactions
TLF	Tables, Listings, and Figures
TOC	Table of Content
U.S.	United States
V	Visit
vac	Vaccination
WHO	World Health Organization

1. BACKGROUND AND RATIONALE

The sponsor (Seqirus, Inc. "Seqirus") is developing a monovalent inactivated influenza vaccine that is adjuvanted with MF59C.1 (MF59, proprietary adjuvant) and uses a surface antigen from a potential pandemic H5N1 virus strain candidate (A/turkey/Turkey/1/2005 (H5N1) NIBRG-23 strain) to be tested in clinical studies.

Currently licensed pandemic and pre-pandemic vaccines are produced using the conventional egg-based manufacturing process. However, in the event of a new pandemic, there will be a dramatic increase of vaccine demand that will overwhelm global production capacity. Moreover, any condition that compromises availability of eggs (e.g. bird flu pandemic) will dramatically reduce production of influenza vaccines. Thus, increasing the availability of influenza vaccine to prevent the next pandemic can be viewed as largely dependent on developing methods of producing influenza vaccines that can avoid the use of eggs as the primary production substrate. Clinical development of the cell culture-derived, MF59-adjuvanted H5N1 (aH5N1c) vaccine helps address the medical need of a safe and effective pandemic and pre-pandemic vaccine.

In dose-ranging Phase 1/2 study V89P1, the aH5N1c vaccines were well tolerated in healthy adults (18-40 years of age), and reactogenicity and safety results of all 12 vaccine groups were similar. No dose-dependent safety issues or vaccine-related long-term effects on safety were observed. Laboratory safety tests performed in a subset of 120 subjects did not raise any safety concerns.

Three Phase 2 studies in separate age populations have been performed evaluating "low dose" (3.75µg HA of H5N1 with 0.125mL MF59) and "high dose" (7.5µg HA of H5N1 with 0.25mL MF59) formulations of aH5N1c:

- V89_04 – adults 18 to <65 years of age.
- V89_11 – children and adolescents 6 months to <18 years of age.
- V89_13 – adults ≥ 65 years of age.

No safety concerns were identified in these Phase 2 studies.

The proposed study is designed to evaluate the safety, immunogenicity and lot-to-lot consistency of aH5N1c vaccine (the "high dose" formulation in the Phase 2 studies) and provide the support required for registration of the product.

The vaccine is given as a two-dose series (on days 1 and 22) and the primary endpoint is measured 21 days after the second dose (day 43).

An interim analysis of immunogenicity will be performed for the current study after Day 43 immunogenicity data are available for all subjects. The objective of the interim

analysis is to provide the information of immunogenicity of study vaccines to the public health authorities to facilitate their planning in case a pandemic were to occur, not for futility or safety.

The final analysis will be performed when all data up to the study end (Day 387, nominally 365 days after the second vaccination) are available.

For further details on the background and rationale of the study, please refer to section 1.0 of the Protocol.

2. OBJECTIVES

2.1 Primary Objectives

Co-Primary Immunogenicity Objectives

To demonstrate lot-to-lot consistency across three consecutively produced lots of aH5N1c vaccine, as assessed by the ratio of geometric mean titers (GMTs) of hemagglutination inhibition (HI) antibody responses to the H5N1 vaccine strain 3 weeks after the second vaccine administration (Day 43) in healthy adult subjects ≥ 18 years of age.

After lot-to-lot consistency is demonstrated, the populations of all H5N1c vaccine recipients will be pooled in order to evaluate immune responses to aH5N1c vaccine according to immunogenicity criteria defined by Center for Biologics Evaluation Research (CBER) guidance 3 weeks after the second vaccine administration (Day 43) as measured by age cohort and by strain-specific HI assay.

Primary Safety Objective

To evaluate the safety and tolerability of aH5N1c vaccine and placebo in healthy adult subjects ≥ 18 years of age.

2.2 Secondary Objectives

Secondary Immunogenicity Objectives

After lot-to-lot consistency is demonstrated, the populations of vaccine recipients administered aH5N1c lots will be pooled, and the following objectives assessed:

- To evaluate immune responses to aH5N1c vaccine according to immunogenicity criteria defined by Committee for Medical Products for Human Use (CHMP) recommendations (CHMP 2003, CHMP 2006) 3 weeks after the second vaccine administration (Day 43) in healthy adult subjects ≥ 18 years of age by age cohort, as measured by strain-specific HI assay.
- To evaluate immune responses to aH5N1c vaccine according to immunogenicity criteria defined by CBER and CHMP recommendations 3 weeks after the first vaccine administration (Day 22), in subjects ≥ 18 years of age by age cohort Both CBER and CHMP criteria will be evaluated separately for each age cohort (18 to < 65 years and ≥ 65 years, and 18 to < 60 years and ≥ 60 years, respectively), as measured by strain-specific HI assay.
- To evaluate immune responses to aH5N1c vaccine 6 months after first vaccine administration (Day 183) in subjects ages ≥ 18 years of age, by age cohort, as measured by strain-specific HI assay.

Table 2-1: Comparison of CHMP and CBER Criteria for Evaluation of Influenza Vaccines

CHMP Criteria	**Adults 18 – <60 Years**	**Adults ≥ 60 Years**
Percentage of subjects with HI antibody titer ≥1:40	> 70%	> 60%
Geometric Mean Ratio	> 2.5	> 2.0
Percentage of subjects with Seroconversion [a]	> 40%	> 30%
CBER Criteria	**Adults 18-64 Years**	**Adults ≥65 Years**
Lower limit of the two-sided 95% CI for percentage of subjects with HI antibody titer ≥1:40	≥ 70%	≥ 60%
Lower limit of the two-sided 95% CI for percentage of subjects with Seroconversion [a]	≥ 40%	≥ 30%

[a] Seroconversion = either a pre-vaccination (baseline) HI titer <1:10 and post-vaccination HI titer ≥1:40, or a pre-vaccination HI titer ≥1:10 and a ≥4-fold increase in post-vaccination HI antibody titer

3. STUDY DESIGN

This is a Phase 3 stratified, randomized, observer-blind, multi-center, placebo-controlled study to evaluate safety, immunogenicity and lot-to-lot consistency of aH5N1c in healthy adult subjects ≥ 18 years of age.

Approximately 3192 subjects will be recruited and randomized at in 1:1:1:1 ratio, stratified by site and age, to receive one of 3 lots of aH5N1c or placebo (Groups A, B, C, and D). The design is summarized in Table 3-1.

Table 3-1: Study Design

Treatment Arm	Schedule of Vaccine Administration	Total Enrolled	Enrolled by Age Group	
			Age 18 to < 65 yrs	Age ≥ 65 yrs
Group A: aH5N1c lot #1	Day 1, Day 22	798	399	399
Group B: aH5N1c lot #2	Day 1, Day 22	798	399	399
Group C: aH5N1c lot #3	Day 1, Day 22	798	399	399
Group D: Placebo	Day 1, Day 22	798	399	399

The 2 doses of vaccine will be administered 3 weeks apart, on Day 1 and Day 22. The co-primary immunogenicity analysis will be based on HI antibody titers collected 3 weeks after the second vaccine administration, on Day 43. Durability of HI antibody response will be evaluated 6 months after the first vaccine administration, i.e. Day 183.

The study duration for each subject will be of approximately 13 months, or approximately 12 months after the second vaccine administration. The study requires that the site be in contact with the subject 17 times over the course of the study, including 5 clinic visits, 4 reminder calls and 8 safety calls during Treatment and Follow-up Periods.

- Treatment Period: Day 1 through Day 42, which includes 2 clinic visits and 4 reminder calls.
- Follow-up Period: Day 43 through Day 387, which includes 3 clinic visits and 8 safety calls.

A detailed schedule and listing of procedures are shown in Table 3-2, Time and Events Table.

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

Table 3-2: Times and Events Table, Scheduled Subject Contacts 1 – 17

Study Periods	Treatment Period						Follow-up Period										
Visit Number (V); Reminder Call (RC) Safety Call (SC); Study Day (D)	V1 D1	RC D3	RC D5	V2 D22	RC D24	RC D26	V3 D43	V4 SC D91	V5 SC D122	V6 SC D152	V7 D183	V8 SC D217	V9 SC D251	V10 SC D285	V11 SC D319	V12 SC D353	V13 D387
Visit Window	Day -5 to Day 1	2-3 days post 1st vac	4-5 days post 1st vac	21-30 days post 1st vac	2-3 days post 2nd vac	4-5 days post 2nd vac	21-30 days post 2nd vac	90 – 100 days post 1st vac	121-131 days post 1st vac	151-161 days post 1st vac	182 – 192 days post 1st vac	216- 225 days post 1st vac	250- 259 days post 1st vac	284-293days post 1st vac	318-327 days post 1st vac	352-361 days post 1st vac	386-395 days post 1st vac
Informed Consent [a]	X																
Medical history [b]	X																
Review of systems [b]	X			X			X				X						X
General physical examination [b]	X																
Symptom-directed physical examination [b]				X			X				X						X
Pregnancy test [c] (Women of childbearing potential)	X			X			X										
Measurement of body temperature (preferably oral) [d]	X			X													
Exclusion/Inclusion criteria [e]	X																
Enrollment and randomization [f]	X																
Serology blood draw (max: 12 mL whole blood) [g]	X			X			X				X						

Study Periods	Treatment Period						Follow-up Period										
Visit Number (V); Reminder Call (RC) Safety Call (SC); Study Day (D)	V1 D1	RC D3	RC D5	V2 D22	RC D24	RC D26	V3 D43	V4 SC D91	V5 SC D122	V6 SC D152	V7 D183	V8 SC D217	V9 SC D251	V10 SC D285	V11 SC D319	V12 SC D353	V13 D387
Visit Window	Day -5 to Day 1	2-3 days post 1st vac	4-5 days post 1st vac	21-30 days post 1st vac	2-3 days post 2nd vac	4-5 days post 2nd vac	21-30 days post 2nd vac	90 – 100 days post 1st vac	121-131 days post 1st vac	151-161 days post 1st vac	182 – 192 days post 1st vac	216- 225 days post 1st vac	250- 259 days post 1st vac	284-293days post 1st vac	318-327 days post 1st vac	352-361 days post 1st vac	386-395 days post 1st vac
Review criteria for repeat vaccination [h]				X													
Study vaccine administered [i]	X			X													
30 minute post-injection adverse event assessment [j]	X			X													
Diary card dispensed (and training on completion) [j]	X			X													
Diary card reminder call [k]		X	X		X	X											
Diary card reviewed and collected [l]				X			X										
Assess all AEs [m]	X			X			X										
Assess local and systemic solicited AEs [l]				X			X										
Assess SAEs, AESIs, medically attended AEs, AEs leading to withdrawal, and NOCD [m]	X			X			X	X	X	X	X	X	X	X	X	X	X
Assess relevant medications [n]	X			X			X	X	X	X	X	X	X	X	X	X	X

Study Periods	**Treatment Period**						**Follow-up Period**										
Visit Number (V); Reminder Call (RC) Safety Call (SC); Study Day (D)	**V1 D1**	**RC D3**	**RC D5**	**V2 D22**	**RC D24**	**RC D26**	**V3 D43**	**V4 SC D91**	**V5 SC D122**	**V6 SC D152**	**V7 D183**	**V8 SC D217**	**V9 SC D251**	**V10 SC D285**	**V11 SC D319**	**V12 SC D353**	**V13 D387**
Visit Window	**Day -5 to Day 1**	**2-3 days post 1st vac**	**4-5 days post 1st vac**	**21-30 days post 1st vac**	**2-3 days post 2nd vac**	**4-5 days post 2nd vac**	**21-30 days post 2nd vac**	**90 – 100 days post 1st vac**	**121-131 days post 1st vac**	**151-161 days post 1st vac**	**182 – 192 days post 1st vac**	**216- 225 days post 1st vac**	**250- 259 days post 1st vac**	**284-293days post 1st vac**	**318-327 days post 1st vac**	**352-361 days post 1st vac**	**386-395 days post 1st vac**
Study Termination [o]																	**X**

Footnotes:

a. See sections 3.2.1 and 12.2 of the Protocol.
b. See section 6.2 of the Protocol.
c. See section 3.5 of the Protocol.
d. See sections 3.2.5.1 and 4.3 of the Protocol.
e. See sections 4.1 and 4.2 of the Protocol.
f. See sections 4.1, 4.2 and 4.4 of the Protocol.
g. See sections 3.2.3 and 3.2.4 of the Protocol.
h. See section 3.1 of the Protocol.
i. See sections 3.2.5.2, 5.1 and 5.3 of the Protocol.
j. See section 3.2.5.3 of the Protocol.
k. See section 3.2.5.4 of the Protocol.
l. See section 3.4.1 of the Protocol.
m. See sections 6.6.1, 6.6.1.1, 6.6.2 and 6.6.3 of the Protocol.
n. See section 5.4 of the Protocol.
o. See section 3.2.5.8 of the Protocol.

4. RANDOMIZATION AND BLINDING

4.1 Method of Group Assignment and Randomization

After an individual has consented to participate in the study and informed consent form (ICF) is signed, a Subject Number will be assigned and screening procedures should be carried out (see section 3.2.2 of the Protocol).

In the event that the individual is determined ineligible for study participation, he/she is considered a "screen failure". The reason for screen failure must be documented in the Screening and Enrollment Log.

After signing the ICF, if an individual is determined to be eligible for study participation, the investigator will enroll the subject and enter the Subject Number into an Electronic Data Capture (EDC) system.

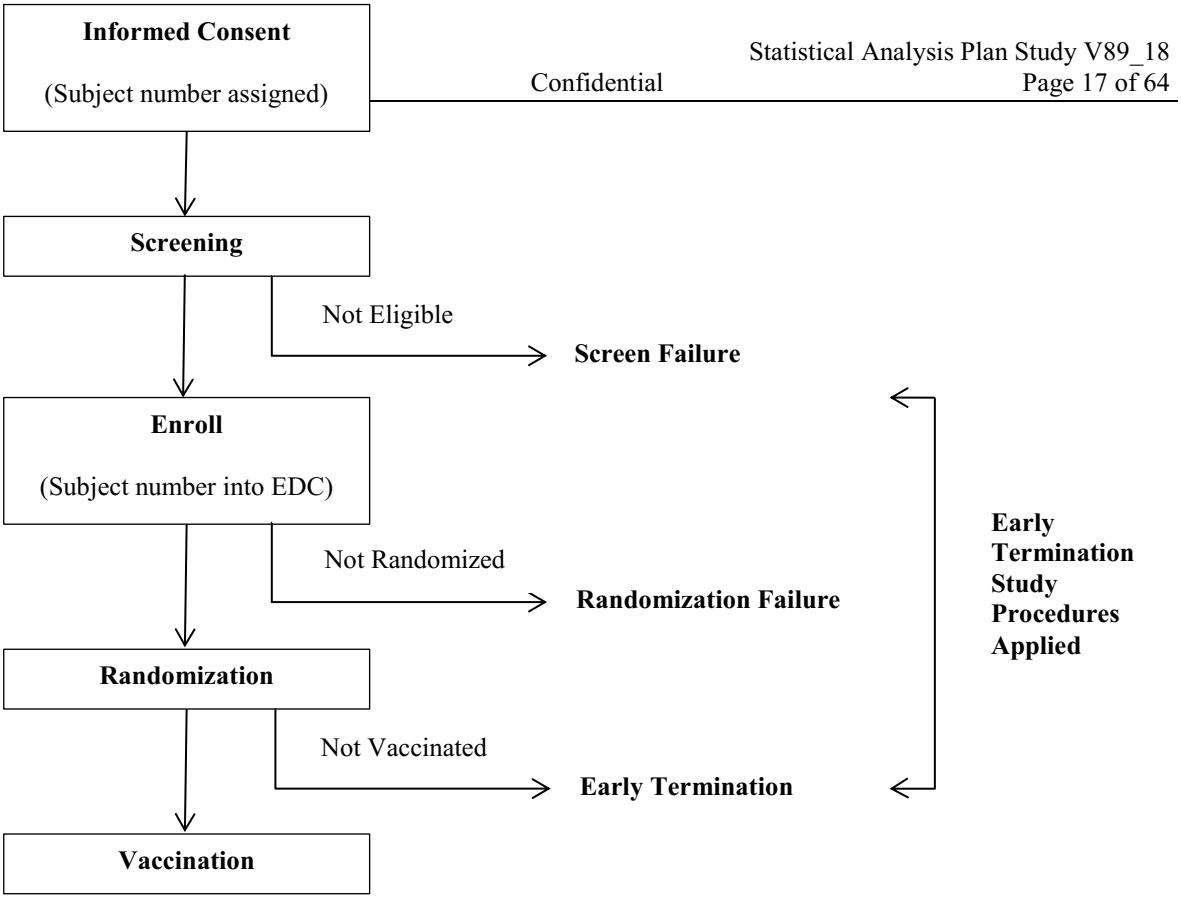
Enrolled subjects will be randomly assigned to the study groups, either one of 3 lots of aH5N1c or placebo, in a pre-specified ratio of 1:1:1:1. Randomization will be stratified by center, and by age cohort (18 to <65 and ≥65 years). A validated randomization system will be used.

After randomization, the Subject Number continues to be used for subject identification for the duration of the study.

If for any reason, after signing the ICF, the subject who is eligible and enrolled fails to be randomized, this is called a randomization failure and the early termination study procedures must be applied. The reason for all randomization failures should be recorded in the Screening and Enrolment 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 3.2.2 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.

The procedure described above is displayed in the Figure 4.1-1.**Figure 4.1-1Figure 4.1-1: Description of Study Procedures**



4.1.1 Definition of Vaccination/Randomization Errors

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

- Subjects vaccinated with a vaccine different from the one assigned at randomization.
- Subjects vaccinated with the correct vaccine but containing a lower volume.
- Subjects randomized into the wrong stratum

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

4.2 Blinding and Unblinding

4.2.1 Randomized Studies

4.2.1.1 Controlling Access to the Study Randomization

Randomization and unblinding will be managed by the PAREXEL Informatics group through Clinphone Interactive Response Technology (IRT). Details of randomization and unblinding procedure in IRT will be provided in the IRT manual.

For an observer-blind study, it is mandatory for the site to have blinded and unblinded staff. The blinded staff will have no access to the randomization or to the vaccine supply, except when the supply is in the blinded kit boxes. Vaccine administration is performed by unblinded site personnel and the subject will not see the vaccine syringe nor be told of the randomization group (aH5N1c or placebo). The unblinded site personnel will not be included in the collection of data nor in the evaluation of the subject.

However, the investigator maintains the right to unblind a subject's treatment at any time if necessary for the subject's safety. Except in the case of medical necessity, a subject's treatment should not be unblinded without the approval of the Sponsor. In such instance of medical emergency, every effort should be made to contact the Sponsor prior to unblinding. In the event that unblinding has occurred, the circumstances around the unblinding (e.g., date and reason) must be documented promptly, and the Sponsor or its delegate notified as soon as possible. Only the principal investigator or delegate should be unblinded to the subject's treatment assignment. Study site personnel and Sponsor personnel directly associated with the conduct of the study should not be unblinded.

If a subject is unblinded during the study, it is to be reported as major protocol deviation, except for subjects unblinded by Pharmacovigilance due to suspected unexpected serious adverse reactions (SUSAR). The unblinding will be documented appropriately. The first-line analysis excludes unblinded subject(s) in immunogenicity statistical analyses based on the per-protocol set (PPS). The unblinded subjects will be included in the full analysis set (FAS) and safety sets.

4.2.1.2 Blinding of Study Vaccine

Study vaccine will be administered in observer blinded fashion (described in section 4.2.1.1).

For this study using IRT, vaccine will be packed using the 'scrambled pack numbering' methodology. All vaccine outer packs will look identical, and are blinded at the kit level. Each pack will be given a unique pack number. IRT will know the content of each pack. Once the unblinded site staff performing the vaccination has opened the outer pack, this person is unblinded for that subject.

4.2.2 Planned Unblinding for Interim Analysis

A formal interim analysis of immunogenicity is planned when Day 43 immunogenicity data are available for all subjects (excluding those lost to follow-up or withdrawn for other reasons). To preserve the integrity of the study, the interim analysis will be handled by an independent statistician and an independent programmer from Clinical Research Organization (CRO) who are not involved in day-to-day study activities and independent from Sponsor. The unblinded protected data snapshots for interim analysis will be restricted to only unblinded individuals responsible for the interim analyses. The interim analysis results will be available to a restricted number of individuals such as select members of Sponsor senior management not directly working on this study, who will be identified prior to the interim analysis. As the results may be transmitted to the Biomedical Advanced Research and Development Authority within the Department of Health and Human Services (HHS/BARDA), Sponsor personnel engaged in discussions with HHS may also be made aware of results. No individuals with access to study results will be involved in day-to-day study activities or decisions regarding individual subject assessments. Investigators, site staff, Sponsor personnel, CRO personnel, and the serology laboratory will remain blinded to the clinical study data and subjects' individual study arm assignment until final data from all termination visits are locked, including both individual subject serology results and serology results by group.

4.2.3 Planned Unblinding for Final Analysis

The study will be fully unblinded after final database lock.

5. SAMPLE SIZE AND POWER CONSIDERATIONS

Sample size/power considerations are included in the study protocol. Technical details including statistical assumptions and software are given in a separate sample size memo authored by the study Biostatistician. In the same document, a statistical co-reviewer verified and documented the sample size/power considerations. This document was completed prior to finalization of the protocol, see Appendix A for reference.

For details please refer to section 7.4.2.3 of the Protocol.

6. DETERMINATION OF PROTOCOL DEVIATIONS

6.1 Definition of Protocol Deviations

Deviations from the protocol will be assessed as 'minor' or 'major'. Major (reportable) protocol deviations (PDs) from the protocol that will lead to the exclusion of the subject or part of the subject's data from the analysis set will be identified a priori. Major PDs are defined as those deviations from the protocol likely to have an impact on the perceived immunogenicity and/or safety of study treatments. The impact of major PDs on the immunogenicity and/or safety results will be investigated by assessing the robustness of the study results. The final determination of major PDs and the exclusion of subjects from each of the analysis populations will be made prior to unblinding the database.

All major PDs will be classified into the following categories, but not all deviations listed below will necessarily be declared a major PD:

- Informed Consent
- Inclusion/Exclusion criteria
- Withdrawal Criteria
- Investigational Product (IP) Admin/Study Treat
- Disallowed Medications
- Adverse Event (AE)/ Serious Adverse Event (SAE)
- Visit Schedule
- Procedure/Tests

Major protocol deviations and any action to be taken regarding the exclusion of subjects or affected data from specific analyses are defined in Table 6.1-1.

Table 6.1-1: Exclusion from Specific Analyses due to Major Protocol Deviations

Major Protocol Deviation	Action to be Taken For Analysis
Subject was not administered any study vaccine	Exclude from all population set
Serological results are not available	Exclude from Full Analysis Set (FAS) Exclude from Per Protocol Set (PPS)
Obvious deviation from laboratory manual or error in laboratory data	Exclude from FAS Exclude from PPS
Subject did not provide any post-vaccination unsolicited safety data	Exclude from Unsolicited Safety Set
Subject did not provide any post-vaccination	Exclude from Solicited Safety Set

Major Protocol Deviation	Action to be Taken For Analysis
solicited safety data	
Randomization failure	Exclude from PPS
Randomization code was broken	Exclude from PPS
Vaccination not according to protocol	Exclude from PPS
Administration of forbidden vaccine	Exclude from PPS
Subject did not meet entry criteria	Exclude from PPS
Subject had contraindication for a subsequent study vaccination but was vaccinated	Exclude from PPS
Administration of prohibited medication	Exclude from PPS
Underlying medical condition prohibited by the protocol	Exclude from PPS
Concomitant infection related to the vaccine which may influence immune response	Exclude from PPS
Did not comply with study vaccination schedule	Exclude from PPS
Did not comply with blood draw schedule	Exclude from PPS

Note: PPS = Per Protocol Set; FAS = Full Analysis Set

The following PD summaries will be provided:

- Number and percentage of subjects with a major protocol deviation by type of deviation and vaccine group

A by-patient listing of protocol deviations will be provided.

6.2 Determination of Protocol Deviations

Prior to unblinding, a PD report will be provided to the Clinical Study Team (CST) consisting of medical, clinical, and operational team members from the Sponsor and CRO for review on an ongoing basis during the study. The PDs review is part of the Data Listing Review process.

After the review, the CST team is responsible for assessing the impact of PDs on the immunogenicity and safety data for study subjects from medical and clinical perspectives. The PDs will be identified and categorized to determine subjects to be excluded from analysis populations according to the PDs specification and Table 6.1-1.

Details of PD review procedure will be provided in the Medical Monitoring Plan and Data Listing Review Manual.

6.3 Exclusions of Individual Values for Safety Analysis

Some local and systemic adverse events (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 6.3-1 below:

Table 6.3-1: Implausible Solicited Adverse Events

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

7. ANALYSIS SETS

7.1 All Enrolled Set

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

7.2 Exposed Set

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

7.3 Full Analysis Set (FAS), Immunogenicity Set

All subjects in the All Enrolled Set who:

- actually receive at least one dose of study vaccination, and
- provide at least one immunogenicity result at relevant timepoints.

  FAS will also be defined by timepoint and objective:

  - FAS-1 will include all subjects who received at least one dose of study vaccine and provide at least one immunogenicity result at Day 1 and Day 43.
  - FAS-2 will include all subjects who received at least one dose of study vaccine and provide at least one immunogenicity result at Day 1 and Day 22.
  - FAS-3 will include all subjects who received at least one dose of study vaccine and provide at least one immunogenicity result at Day 1 and Day 183.

In case of vaccination error, subjects in the FAS will be analyzed "as randomized" (i.e., according to the vaccine a subject was designated to receive, which may be different from the vaccine the subject actually received). Any subject who received the wrong vaccination will not be excluded from the FAS.

Subjects randomized in the wrong stratum will be analyzed in the stratum in which they were randomized (i.e., analyze subject in wrong stratum).

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

7.4 Per Protocol Set (PPS), Immunogenicity Set

All subjects in the FAS Immunogenicity who:

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

- Have no major protocol deviations leading to exclusion (see section 6.1) as defined prior to unblinding / analysis.
- Are not excluded due to other reasons (e.g. subjects who withdrew informed consent) defined prior to unblinding or analysis (see section 6.2)

In case of vaccination error, subjects in the PPS will be analyzed "as randomized" and the subject who received the wrong vaccination will be excluded from the PPS. If a subject receives a vaccine from the wrong kit number, but the same as the one the subject was randomized to, the subject will not be removed from the PPS.

Subjects randomized in the wrong stratum will be excluded from the PPS.

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

Similarly to FAS, PPS will also be defined per objective and timepoint:

- PPS-1 will include all subjects who are in FAS-1 and correctly received study vaccine, have no major protocol deviations leading to exclusion and are not excluded due to other reasons as defined prior to unblinding / analysis.
- PPS-2 will include all subjects who are in FAS-2 and correctly received study vaccine, have no major protocol deviations leading to exclusion and are not excluded due to other reasons as defined prior to unblinding / analysis.
- PPS-3 will include all subjects who are in FAS-3 and correctly received study vaccine, have no major protocol deviations leading to exclusion and are not excluded due to other reasons as defined prior to unblinding / analysis.

Exclusions will be considered by objective/time point, i.e., sometimes not all data of a subject but only part of the subject's data will be removed from the PPS analysis.

7.5 Safety Set

Solicited Safety Set

All subjects in the Exposed Set who have gone through any assessment of local and systemic site reaction and/or assessment of any use of analgesics/antipyretics.

Unsolicited Safety Set

All subjects in the Exposed Set who have gone through any adverse event assessments i.e., a subject does not have to have any adverse events to be included in this population.

Overall Safety Set

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

Subjects providing only 30 minutes post-vaccination safety data will also be reported separately in a 30-minute post-vaccination safety analysis.

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).

Subjects randomized in the wrong stratum will be reassigned to the correct stratum and will be analyzed using corrected stratum for all safety sets (i.e., solicited safety set, unsolicited safety set and overall safety set). If a subject is unblinded during the study, he/she will be included in all the safety sets.

7.5.1 Restricted Safety Set

Not applicable.

7.6 Other Analysis Set

Not applicable.

8. GENERAL ISSUES FOR STATISTICAL ANALYSES

8.1 Adjustment for Covariates

Adjusted estimates of geometric mean titers (GMTs), and their associated 95% confidence intervals (CIs) at Day 43 will be determined using analysis of covariance (ANCOVA) with factors for lot, age groups (18 to <65, ≥ 65), center and a covariate for the effect defined by the log-transformed pre-vaccination antibody titer. Influenza-antibody GMTs (Day 43), associated two-sided 95% CIs and median, minimal, and maximal area/titer values will be determined and presented together with N (number of subjects), by lot (for the first primary objective).

This model will include only immunogenic data from the 3 lots of aH5N1.

If the statistical model does not converge due to the factor "center", a model without center effect will be fitted instead.

The placebo group titers/concentrations will be summarized without any adjustment (i.e. unadjusted GMTs and percentages).

Binary data tables will show adjusted percentages and adjusted "between-group" differences.

8.2 Handling of Dropouts, Missing Data

First-line analyses will be without missing values.

To minimize the effect of dropouts and missing data the study period will be divided into time intervals for statistical analysis of immunogenicity.

8.2.1 Safety Data

For unsolicited adverse events, the entire study period will be divided into the following intervals: Day 1-22, Day 23-43, Day 1-43, Day 44-387, Day 1-387.

For solicited adverse events, the solicited study period 30 min - Day 7 after each vaccination will be divided into: 30 min, Day 1-3 (with and without the 30-minute interval), Day 4-7, and Day 1-7 (with and without the 30-minute interval).

No imputation of missing solicited or unsolicited AEs will be used. The percentage of subjects with missing solicited AE assessments and missing safety phone calls or safety assessment, including subjects who discontinued the study, 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 primary objective of lot-to-lot consistency will be analyzed using the PPS and the other primary objective will be analyzed using the PPS, and repeated with the FAS if the numbers of subjects between PPS and FAS differ by >5%.

8.2.3 Efficacy Data

Not applicable.

8.3 Multicenter Studies

Vaccine group effects will be investigated first using a linear model which allows for center differences, but does not consider vaccine-by-center interaction, i.e., the model only considers effects for center and vaccine. A center effect will be included in all analyses of the primary and secondary objectives.

If the statistical model does not converge due to the factor "center", a model without center effect will be fitted instead.

8.4 Multiple Comparisons and Multiplicity

For lot-to-lot consistency in the statistical analysis of the primary objectives, a combined equivalence hypothesis will be tested.

There is no need for adjustment for multiplicity.

8.5 Immunogenicity Subsets

Not applicable.

8.6 Subgroups

Subgroup analysis will not be applied to the primary objective of lot-to-lot consistency. Using the FAS and PPS, analyses of other primary objectives will be repeated and stratified by

- Baseline HI titer (<1:10 vs. ≥1:10)
- With and without seasonal influenza vaccine in the last 12 months
- Sex (Male vs. Female)

- Race (American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or other Pacific Islander, White, Other)
- Ethnicity (Hispanic or Latino, Not Hispanic or Latino, Not Reported, Unknown)
- Age per CBER (18 to <65 vs. ≥65 years)
- Age per CHMP (18 to <60 vs. ≥60 years)
- Age (18 – 49, 50 – 64, 65 – 74, ≥75 years)
- Center (as applicable)

For details regarding the analyses by center please see section 8.3 above. Analyses by center will be presented in Appendix 16.1.9 of the CSR.

Analyses of safety data will be repeated and stratified by each of the following: age per CBER (18 to <65 vs. ≥65 years) and age per CHMP (18 to <60 vs. ≥60 years), sex and race for Solicited and Unsolicited AEs. The additional age group (18 - <50, 50 - <65, 65 - <70, and ≥70 years) will be applied to the Solicited AEs. Stratification by age per CBER (18 to <65 vs. ≥65 years) and age per CHMP (18 to <60 vs. ≥60 years) will only be applied to the SAE, NOCD, AESI, AEs leading to death, premature termination, and dose modification.

8.7 Derived and Computed Variables

Demographics

Age will be calculated in years using the following formula:

Age (years) = (Date of Visit 1 – Date of Birth + 1) / 365.25, round to smallest integer

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

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

Immunogenicity

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

Titer greater or equal to a given threshold is defined as binary variable for non-missing values as:

= 1, if the titer is superior or equal to the given threshold

= 0, otherwise

HI antibody titer ≥1:40 is defined as binary variable for subjects with non-missing values as:

= 1, if achieving a HI antibody titer ≥1:40

= 0, otherwise

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

= 1, if there is a post-vaccination titer ≥ 1:40 for pre-vaccination titer < 1:10

= 1, if there is 4-fold increase (post-vaccination) from pre-vaccination titer ≥1:10

= 0, otherwise

Geometric Mean Titer/Concentration

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/concentrations.

Geometric Mean Ratio

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

The GMR will be calculated using the following formula:

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

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

Duration in the Study

Duration in the study is defined in days as:

Last visit date (visit 13)[a] – Enrollment date (visit 1) + 1

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

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

Solicited Adverse Events

For details see section 13.2.

Unsolicited Adverse Events

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

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

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

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

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

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

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

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

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

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

Safety Laboratory Data

Not applicable

Pre-study, Concomitant and Post-Study Medications

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

A **post-study medication** is a medication used only after study termination (i.e. medication start date > study termination date). This will not be collected in the clinical database and will not be reported in the CSR.

All other medications are **concomitant**.

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

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

8.8 Analysis Software

All analyses will be performed using SAS® Software version 9.1 or higher.

8.9 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 log10-transformed. GMTs and their 95% CIs are computed by exponentiating (base 10) the least squares means and 95% CIs of the log10 titers (obtained from a fitted model as appropriate).

9. STUDY SUBJECTS

9.1 Disposition of Subjects and Withdrawals

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

The time the subjects are under observation will be summarized by vaccine and overall using summary statistics (mean, standard deviation (SD), minimum, median, maximum).

10. DEMOGRAPHICS AND OTHER BASELINE CHARACTERISTICS

10.1 Demographics

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

The frequencies and percentages of subjects by sex, ethnic origin, race, and previous influenza vaccination (in the past 12 months) will be presented by vaccine lot/group and overall. Demographic data will be tabulated for the All Enrolled, PPS, and Overall Safety Set. If the percentage of subjects excluded from the PPS is greater than 5%, the demographic data will be tabulated for the FAS as well.

10.2 Medical History

The frequencies and percentages of subjects with medical history will be presented by MedDRA system organ class and preferred term, by vaccine lot/group and overall. Medical history data will be tabulated for the All Enrolled, PPS, and Overall Safety Set. If the percentage of subjects excluded from the PPS is greater than 5%, the medical history data will be tabulated for the FAS as well.

11. IMMUNOGENICITY ANALYSIS

11.1 Blood Samples

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

11.2 Primary Objectives Analysis

For lot-to-lot consistency, the following equivalence hypotheses will be tested simultaneously:

H_0: $(\mu_{lot\ A} - \mu_{lot\ B}) \leq -0.176$ or $(\mu_{lot\ A} - \mu_{lot\ B}) \geq 0.176$ or

$(\mu_{lot\ A} - \mu_{lot\ C}) \leq -0.176$ or $(\mu_{lot\ A} - \mu_{lot\ C}) \geq 0.176$ or

$(\mu_{lot\ B} - \mu_{lot\ C}) \leq -0.176$ or $(\mu_{lot\ B} - \mu_{lot\ C}) \geq 0.176$

vs.

H_1: $(\mu_{lot\ A} - \mu_{lot\ B}) > -0.176$ and $(\mu_{lot\ A} - \mu_{lot\ B}) < 0.176$ and

$(\mu_{lot\ A} - \mu_{lot\ C}) > -0.176$ and $(\mu_{lot\ A} - \mu_{lot\ C}) < 0.176$ and

$(\mu_{lot\ B} - \mu_{lot\ C}) > -0.176$ and $(\mu_{lot\ B} - \mu_{lot\ C}) < 0.176$

Here, H_1 refers to the alternative hypothesis of pairwise equivalence (consistency) transformed to the log10 scale. Accordingly, $\mu_{lot\ A}$, $\mu_{lot\ B}$, and $\mu_{lot\ C}$ denote the means of log10-transformed Day 43 titers of the corresponding lot groups. The lot-to-lot consistency will be claimed if the two-sided 95% CIs of all the three pairwise comparisons are within the equivalence ranges. Significance level to all these tests is α = 5%, which needs no adjustment for multiplicity as all hypotheses have to be rejected (union-intersection principle).

The primary analysis population for testing the null hypotheses above will be the Per Protocol Set (PPS). If the percentage of subjects excluded from the PPS for analysis of immunogenicity is greater than 5%, a supporting analysis based on the FAS will be performed to compliment the analysis based on the PPS.

For evaluating immune responses to aH5N1 vaccine (percentage of subjects with HI titer ≥ 1:40) according to CBER criteria:

- The lower bound of the adjusted 2-sided 95% CI for the percentage of subjects achieving an HI antibody titer ≥1:40 should meet or exceed 70% and 60% respectively for the two different age cohorts (18 to <65 years of age and ≥65 years of age), the following hypothesis will be tested:

  H_0: $(\pi_i - \pi_0) \leq 0$ vs. H_1: $(\pi_i - \pi_0) > 0$

  Here, H_1 refers to the alternative hypothesis. π_0 denotes the CBER threshold for proportion of subjects with a HI titer ≥1:40 (π_0=0.7 for subjects 18 to <65 years of age and π_0=0.6 for subjects ≥65 years of age).

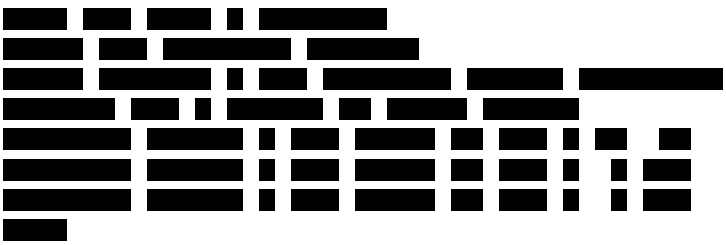
To meet the CBER criteria, proportion for both HI antibody titer ≥ 1:40 (for both age groups) should be met at significance levels of α = 5%. Adjustment for multiplicity is not needed as all hypotheses have to be rejected i.e., rejection region for the composite hypothesis is an intersection of rejection regions for all individual hypotheses.

Adjusted estimates of geometric mean titers (GMTs), and their associated 95% CIs at Day 43 will be determined using analysis of covariance (ANCOVA) with factors for lot, age groups (18-64, ≥65), center and a covariate for the effect defined by the log-transformed pre-vaccination antibody titer. Influenza-antibody GMTs (Day 43), associated two-sided 95% CIs and median, minimal, and maximal area/titer values will be determined and presented together with N (number of subjects), by lot (for the first primary objective). This model will include only immunogenicity data from the 3 lots of aH5N1.

Statistical analyses will be performed on the logarithmically (base 10) transformed titer values, while median, minimum, and maximum values will be obtained on the actual titer values.

To assess equivalence of lots, the limit of the 95% CI will be compared with the pre-specified thresholds.

The following SAS® code will be used for the ANCOVA model:



where Ab_post represents the log-transformed antibody value of the immunogenicity variable at a given post baseline timepoint, trt indicates the vaccine group, agegroup the age group (18-64, ≥65), center the site, baseline the log-transformed antibody baseline value of the immunogenicity variable.

Binary data (proportion of subjects with HI titer ≥ 1:40) will be analyzed using log-linear models with a factor for dose group adjusted for center, at Day 43. Proportions and two sided 95% CIs will be calculated based on these models. Models might be simplified in case of convergence problems.

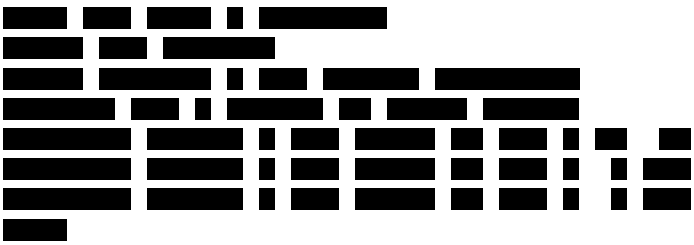
The following SAS® code will be used for the analysis of binary data:

The analysis will be based on the PPS. If the percentage of subjects excluded from the PPS for analysis of immunogenicity is greater than 5%, a supporting analysis based on the FAS will be performed to compliment the analysis based on the PPS.

11.3 Secondary Objectives Analysis

Adjusted estimates of geometric mean titers (GMTs), and their associated 95% CIs at Days 1, 22, 43 and 183 will be determined using analysis of covariance (ANCOVA) with factors for vaccine dose group and center, by age cohort. GMTs at Days 22, 43 and 183 will be adjusted by the pre-vaccination antibody titer. Influenza-antibody GMTs, associated two-sided 95% CIs and median, minimal, and maximal area/titer values will be determined and presented together with N (number of subjects), by vaccine group in both age cohorts (for CBER and CHMP objectives). Statistical analyses will be performed on the logarithmically (base 10) transformed titer values, while median, minimum, and maximum values will be obtained on the actual titer values.

The following SAS® code will be used for the ANCOVA model:



where Ab_post represents the log-transformed antibody value of the immunogenicity variable at a given post baseline timepoint, trt indicates the vaccine group, center the site, baseline the log-transformed antibody baseline value of the immunogenicity variable.

Binary data (proportion of subjects with HI titer ≥ 1:40 and proportions of subjects achieving seroconversion) will be analyzed using log-linear models with a factor for treatment group adjusted for center, at Days 1, 22, 43 and 183. Proportions and two-sided 95% CIs will be calculated based on these models. Models might be simplified in case of convergence problems. The same statistical model as the one used for the primary objective will be applied.

To assess CBER criteria, summarize Table 11-1 below, the lower limit of the 95% CI of the two endpoints (proportion of Subjects with HI titer ≥ 1:40 and proportions of subjects achieving seroconversion) will be compared with the pre-specified thresholds, in the two age cohorts (18-64, ≥ 65 years). Similarly, CHMP criteria will be assessed.

Table 11-1: Comparison of CHMP and CBER Criteria for Evaluation of Influenza Vaccines

CHMP Criteria	**Adults 18 – <60 Years**	**Adults ≥ 60 Years**
Percentage of subjects with HI antibody titer ≥1:40	> 70%	> 60%
Geometric Mean Ratio	> 2.5	> 2.0
Percentage of subjects with Seroconversion [a]	> 40%	> 30%
CBER Criteria	**Adults 18-64 Years**	**Adults ≥65 Years**
Lower limit of the two-sided 95% CI for percentage of subjects with HI antibody titer ≥1:40	≥ 70%	≥ 60%
Lower limit of the two-sided 95% CI for percentage of subjects with Seroconversion [a]	≥ 40%	≥ 30%

[a] Seroconversion = either a pre-vaccination (baseline) HI titer <1:10 and post-vaccination HI titer ≥1:40, or a pre-vaccination HI titer ≥1:10 and a ≥4-fold increase in post-vaccination HI antibody titer

In addition, results collected at Day 22 will also be evaluated against the CBER criteria for both age cohorts. Results collected at Day 22 will also be evaluated against the CHMP criteria.

The analysis will be based on the PPS. If the percentage of subjects excluded from the PPS for analysis of immunogenicity is greater than 5%, a supporting analysis based on the FAS will be performed to compliment the analysis based on the PPS.

11.4 Exploratory Objectives Analysis

Not applicable.

12. EFFICACY ANALYSIS

12.1 Primary Objectives Analysis

Not applicable.

12.2 Secondary Objectives Analysis

Not applicable.

12.3 Exploratory Objectives Analysis

Not applicable.

13. SAFETY ANALYSIS

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

- Vaccine exposure.
- Solicited local and systemic adverse events.
- Unsolicited adverse events.

13.1 Analysis of Extent of Exposure

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

13.1.1 Safety Completeness Analysis

Solicited adverse events

The safety completeness analysis on solicited adverse events aims to identify subjects who completed diary cards, irrespective of severity. The analysis will show the number of subjects with *valid data* by solicited adverse event 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:

- The frequencies of subjects who provide diary cards by vaccine group and collection method.
- For each solicited adverse event, the frequencies of subjects with valid data will be presented by vaccine group and timepoint: 30 min, Day 1-3 (with and without the 30-minute interval), Day 4-7, and Day 1-7 (with and without the 30-minute interval).
- For each type of solicited adverse event (local, systemic) and indicators of solicited adverse events, such as analgesic use the frequencies of subjects with valid data by vaccine group, aggregated over time points: 30 min, Day 1-3 (with and without the 30-minute interval), Day 4-7, and Day 1-7 (with and without the 30-minute interval).
- For each solicited adverse event, the frequencies of subjects with valid data by vaccine group, aggregated over time points: 30 min, Day 1-3 (with and without the 30-minute interval), Day 4-7, and Day 1-7 (with and without the 30-minute interval).

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' analysis set.

13.2 Solicited Local and Systemic Adverse Events

Only solicited local and systemic adverse events reported in the diary card will be analyzed. Implausible measurements will not be taken into consideration in the analysis but listed in the Appendix of the CSR (see section 6.3 of this document).

Solicited adverse events will be collected at 30 minutes, Day 1 (after 30 minutes) and then daily until Day 7 using structured diaries. The analyses of solicited adverse events will be done separately for 30 minutes and based on the intervals: Day 1-3 (with and without the 30-minute interval), Day 4-7, and Day 1-7 (with and without the 30-minute interval). In addition solicited adverse events 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:

Grade 0 (<25 mm), any (25-50 mm [Grade I], 51-100 mm [Grade II], >100 mm [Grade III]).

Injection site pain and systemic AEs (except fever) occurring for the 7 days including each vaccination will be summarized according to "mild", "moderate" or "severe".

Each solicited local and systemic AE will also be further summarized as "none" versus "any".

Body temperature will be broken down by route of measurement according to the recommendations of the Brighton collaboration and will be summarized according to the 2 schemes described below:

- by 0.5 °C increments: <36.0, ≥36.0-<36.5, ≥36.5-<37.0, ≥37.0-<37.5, ≥37.5-<38.0, ≥38.0-<38.5, ≥38.5-<39.0, ≥39.0-<39.5, ≥39.5-<40.0, ≥40.0°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 an indicator of a systemic adverse event.

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

1. Daily reports of subjects with solicited adverse events.
2. Time of first onset of solicited adverse events (excluding 30 min measurement).

3. Solicited adverse events, maximum event severity by event and interval Day 1-3, Day 4-7, and Day 1-7, each without 30 min.
4. Duration of solicited adverse events.
5. Solicited adverse events and indicators of solicited adverse events, occurrence of at least one event by category (local, systemic) and interval Day 1-3, Day 4-7 and Day 1-7, 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 adverse events in the interval of interest will be considered. Subjects without plausible data (i.e. missing values or reported as "Not done/unknown" and implausible values) will be removed from the denominator to prevent a downward bias (towards zero).

Level 1: Daily reports of solicited adverse event

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

Level 2: Time of first onset of solicited adverse events

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

Table 13.2-1: Example for Time to First Onset of Solicited Adverse Events

Vaccination	Subject Number	Day 1[a]	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	…	None
2	001	None	None	None	None	…	Not done
	002	None	Mild	Mild	Missing	…	Missing
	003	Severe	None	Mild	Missing	…	None
	004	Missing	Missing	Missing	Severe	…	Mild

[a] Exclude 30 minutes after vaccination.

For each vaccination the first onset of the adverse event will be used for each subject. For any vaccination the worst adverse event across all vaccinations per time point will be used. Note, 'not done' is treated identical to 'missing'. A mock-up table is shown in Table 13.2-2 below.

Table 13.2-2: Time to First Onset of Solicited Adverse Events

Vaccine group A

Vaccination	Adverse event		Number (%) of Subjects					
			DAY 1[a] (N=4)	DAY 2 (N=4)	DAY 3 (N=4)	DAY 4 (N=4)	…	DAY 7 (N=4)
1	XY	n	4	4	4	4	…	3
		ANY	3 (75.0%)	1 (25.0%)	0 (0%)	0 (0%)	…	0 (0%)
		Mild	2 (50.0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Moderate	1 (25.0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Severe	0 (0%)	1 (25.0%)	0 (0%)	0 (0%)	…	0 (0%)
2	XY	n	3	3	3	2	…	2
		ANY	1 (33.3%)	1 (33.3%)	0 (0%)	1 (50.0%)	…	0 (0%)
		Mild	0 (0%)	1 (33.3%)	0 (0%)	0 (0%)	…	0 (0%)
		Moderate	0 (0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Severe	1 (33.3%)	0 (0%)	0 (0%)	1 (50.0%)	…	0 (0%)
ANY	XY	n	4	4	4	4	…	3
		ANY	3 (75.0%)	1 (25.0%)	0 (0%)	0 (0%)	…	0 (0%)
		Mild	2 (50.0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Moderate	0 (0%)	0 (0%)	0 (0%)	0 (0%)	…	0 (0%)
		Severe	1 (25.0%)	1 (25.0%)	0 (0%)	0 (0%)	…	0 (0%)

N: no. of subjects with data at a time point across all vaccinations.
n: no. of subjects with data at a time point for that specific vaccination.
[a] Exclude 30 minutes after vaccination.

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

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

Level 4: Number of days with solicited adverse events

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

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

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

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

Medications to treat or prevent pain or fever will be summarized by frequencies and percentages of subjects reporting use of the medications by interval 30 min, Day 1 – 3 (excluding 30 minutes after vaccination), Day 4 – 7, Day 1 – 7 (excluding 30 minutes after vaccination).

For details please refer to section 7.1.1 of the Protocol.

13.3 Unsolicited Adverse Events

The first-line analysis will use unsolicited adverse event data from all reporting sources combined. A second-line analysis will encompass the analysis of unsolicited adverse events by source.

All the unsolicited adverse events occurring during the study, judged either as probably related, possibly related, or not related to vaccination by the investigator, will be recorded. The original verbatim terms used by investigators to identify adverse events in the case report forms (CRFs) will be mapped to preferred terms using the MedDRA dictionary. The unsolicited adverse events will then be grouped by MedDRA preferred terms into frequency tables according to system organ class. Adverse events judged by the investigator as at least possibly related to study vaccine will be summarized by vaccine group, according to system organ class and preferred term within system organ class. When an unsolicited adverse event occurs more than once for a subject, the maximal severity and strongest relationship to the vaccine group will be counted.

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

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

- Onset between Day 1 and Day 22.
- Onset between Day 23 and Day 43.
- Onset between Day 1 and Day 43.
- Onset between Day 44 and study termination.
- Onset between Day 1 and study termination.

The analysis of unsolicited adverse events comprises the following categories:

- Any unsolicited adverse event.
- Possibly or probably related unsolicited adverse events.
- Unsolicited adverse events leading to death.
- Serious adverse events.
- Possibly or probably related serious adverse event.
- Unsolicited adverse events leading to premature withdrawal from study.
- Unsolicited adverse events leading to dose change in study vaccination.
- Unsolicited adverse events leading to new onset of chronic disease (NOCD).
- Unsolicited adverse events of special interest (AESI), see Appendix A of the protocol
- Medically attended adverse events.

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

13.4 Clinical Safety Laboratory Investigations

Not Applicable.

13.5 Concomitant Medication

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

14. INTERIM ANALYSIS

14.1 Interim Analysis

The interim analysis will be performed on serology data, i.e., serum HI titer results for all subjects through the Day 43 visit (excluding those lost to follow-up or withdrawn for other reasons), by independent individuals who are not involved in clinical study conduct or any day-to-day study activities. The unblinded results will be provided to restricted unblinded individuals only i.e., select members of Seqirus senior management not directly working on this study, who will be identified prior to the interim analysis. The unblinded results can be transmitted to HHS/BARDA prior to reporting the complete study (all subjects through day 387 post-dose). This analysis will minimally include demographic, baseline characteristics, medical history, vaccination administration, and immunogenicity (HI titer). The unblinded results will not be depicted as individual subject results; results will be presented by treatment arm only.

The interim analysis will occur after all necessary Day 43 data are available, i.e., approximately 11 months prior to reporting the complete study. The interim analysis of immunogenicity will be performed on cleaned serology data, however the snapshot of other clinical data may not be cleaned at the time of interim analysis, and will represent the final results through day 43. There are no plans to re-assay any serum samples obtained from day 1 to day 43. Day 183 immunogenicity testing will be performed separately. The lot-to-lot analysis will be performed with data from subjects in the PPS and the analyses for seroconversion and titer $\geq$ 1:40 will be performed with data from subjects in the PPS (and possibly the FAS, if the numbers of subjects between PPS and FAS differ by >5%) definable with the clinical data available at the time of this snapshot. The analysis performed at interim analysis will be refreshed at the final analysis on the fully cleaned and locked database.

The objective of the interim analysis is not for futility or safety concerns, hence the study will not be stopped based on the results of the interim analysis.

14.1.1 Futility Analysis

Not Applicable.

15. DATA MONITORING COMMITTEES

A data monitoring committee (DMC) will not be constituted for this study because the past studies with aH5N1 have shown no safety signals requiring monitoring by a DMC and there are planned monitoring safety procedures that can identify important safety issues on a real-time basis. Hence, there is no DMC planned in this study.

16. PEER REVIEW

The type of peer review required for each output is to be identified by the study Biostatistician and Statistical Programmer (SP). Analyses/Outputs requiring statistical peer review correspond usually to the analyses of the primary and secondary objectives, and data conversion programs. Peer review of these analyses should be performed in accordance with the applicable procedures for validation of SAS programs used in clinical data analysis.

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

- Primary immunogenicity analysis (Lot-to-lot analysis, Ratio of GMTs of two consecutive lots).

The following programs are identified as key programs to be peer reviewed by a second SP:

- Protocol deviations.
- Summary of GMTs and associated confidence interval (co-primary analysis).
- Summary and percentage of Subjects achieving HI titer of ≥ 1:40, seroconversion, and associated confidence intervals (co-primary analysis).

17. LIST OF FINAL REPORT TABLES, LISTINGS AND FIGURES

For the complete list of tables, listings and figures (TLFs), please refer to the post-text TLF shell document.

18. LAYOUT SPECIFICATIONS FOR TABLES, LISTINGS AND FIGURES

All TLFs will include a header with the following components, e.g.:

Seqirus Inc.	Vaccine: aH5N1c
Final Report: Study V89_18	Phase III, adults ≥18 yr

The post-text TLFs shells will be created as the layouts of how tables, listings, and figures will be presented.

19. REFERENCES

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

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

Committee for Medicinal Products for Human use (CHMP, 2006). Guideline on clinical evaluation of new vaccines. EMEA/CHMP/VWP/164653/2005

U.S. Department of Health and Human Services, Food and Drug Administration, CBER (2007): Guidance for Industry; Clinical Data Needed to Support the Licensure of Pandemic Influenza Vaccines

20. APPENDIX A

20.1 Documentation of Sample Size Calculation V2.0 Dated 17Jun2014

Biostatistics & Statistical Reporting

V89_18

Documentation of Sample Size Calculation

Author(s):	[redacted]
Document type:	Sample Size Documentation
Document status:	Final
Release date:	17-JUN-2014
Version	2
Number of pages:	5

1 Information to enable the reproduction of the sample size

Co- Primary Immunogenicity Objectives

To demonstrate lot-to-lot consistency across three consecutively produced lots of aH5N1c vaccine, as assessed by the ratio of geometric mean titers (GMTs) of hemagglutination inhibition (HI) antibody responses to the H5N1 vaccine strain 3 weeks after the second vaccination (day 43) in healthy adult subjects ≥ 18 years of age.

To evaluate immune responses to aH5N1c vaccine according to immunogenicity criteria defined by CBER recommendations 3 weeks after the second vaccine administrations (Day 43) as measured by age cohort and by strain-specific HI assays for the pooled study population of aH5N1c vaccine recipients. This evaluation will be carried out after the lot-to-lot consistency is demonstrated.

Primary Safety Objective

To evaluate the safety and tolerability of aH5N1c vaccine and placebo in healthy adult subjects ≥ 18 years of age.

1.1 2.2 Secondary Objectives

Secondary Immunogenicity Objectives

After lot-to-lot consistency is demonstrated, the aH5N1c lots will be pooled, and the following objectives assessed:

- To evaluate immune responses to aH5N1c vaccine according to immunogenicity criteria defined by CHMP recommendations (CHMP 2003, CHMP 2006) 3 weeks after the second vaccine administration (Day 43) in healthy adult subjects ≥ 18 years of age by age cohort*, as measured by strain-specific HI assay.
- To evaluate immune responses to aH5N1c vaccine according to immunogenicity criteria defined by CBER and CHMP recommendations 3 weeks after the first vaccine administration (Day 22), in subjects ≥ 18 years of age by age cohort*, as measured by strain-specific HI assay.
- To evaluate immune responses to aH5N1c vaccine 6 months after first vaccine administration (Day 183) in subjects ages ≥ 18 years of age, by age cohort, as measured by strain-specific HI assay.

* Both CBER and CHMP criteria will be evaluated separately for each age cohort (18 to <65 years and ≥ 65 years, and 18 to <60 years and ≥ 60 years respectively).

1.2 Hypotheses

Primary Objectives

For lot-to-lot consistency, the following equivalence hypotheses will be tested simultaneously:

H_0: (μlot A - μlot B) $\leq$ -0.176 or (μlot A - μlot B) $\geq$ 0.176 or
(μlot A - μlot C) $\leq$ -0.176 or (μlot A - μlot C) $\geq$ 0.176 or
(μlot B - μlot C) $\leq$ -0.176 or (μlot B - μlot C) $\geq$ 0.176

vs.

H_1: (μlot A - μlot B) > -0.176 and (μlot A - μlot B) < 0.176 and
(μlot A - μlot C) > -0.176 and (μlot A - μlot C) < 0.176 and
(μlot B - μlot C) > -0.176 and (μlot B - μlot C) < 0.176

Here, H_1 refers to the alternative hypothesis of pairwise equivalence (consistency) transformed to the log10 scale. Accordingly, μlot A , μlot B, and μlot C denote the means of log10-transformed Day 43 titers of the corresponding lot groups. The lot-to-lot consistency will be claimed if the 2-sided 95% CIs of all the three pairwise comparisons are within the equivalence ranges. Significance level to all these tests is α = 5%, which needs no adjustment for multiplicity as all hypotheses have to be rejected (intersection-union test problem).

For CBER criteria HI antibody titer $\geq$1:40 should meet or exceed 70% and 60% respectively for the two different age cohorts (18- <65 years of age and $\geq$65 years of age) the following hypothesis will be tested:

H_0: $(\pi_i-\pi_0) \leq 0$ vs. H_1: $(\pi_i-\pi_0) > 0$

Here, H_1 refers to the alternative hypothesis. π_0 denotes the threshold for proportion of subjects with a HI titer $\geq$1:40 (π_0=0.7 for subjects 18 to <65 years of age and π_0=0.6 for subjects $\geq$65 years of age). Significance levels to these tests is α = 5% which needs no adjustment for multiplicity as all hypothesis have to be rejected (intersection union problem).

1.3 Sample Size Calculation

Primary Objectives

Data observed in previous NVD studies in similar sets (V89_04 in subjects 18-64 years of age; V89_13 in subjects > 65 years of age) showed a variability of the post vaccination titers ranging from 0.75 to 0.88 (in the log scale). With the proposed sample size, assuming a standard deviation of 0.85 for the log10 antibody titers (for each vaccine lot), approximate pairwise equivalence of factor 1 and independency, a single equivalence test based on 718 subjects per lot group has a power of 95%. The resulting overall power is approximately 86%, because the total number of comparisons is three. To account for dropouts (approximately 10%), a total of n=798 per lot should be recruited.

Data observed in previous NVD studies in similar populations (V89_04 in subjects 18-64 years of age; V89_13 in subjects > 65 years of age) showed a proportion of 86% of subjects aged 18 to <65 years and a proportion of 81% of subjects aged 65 years and older achieving an HI antibody titer of ≥1:40. With a sample size of 1077 evaluable subjects from the pooled lots there is an overall power of approximately 98% to achieve the CBER criteria (HI antibody titer ≥1:40 for at least 70% of the subjects aged 18 to <65 years, and for at least 60% of the subjects aged 65 years and older). To account for dropouts (approximately 10%), a minimum sample size of n=1197 for all lots combined are needed.

1.4 Software and Directories

	Modify / delete whatever is applicable
Sample size package used	1) Nquery
	2) screenshots added below
Method used, with reference (if deemed necessary)	1) Two-group t-test of equivalence of means (equal n's); One group X^2 test that proportion equals user specified value (normal approximation)
Historical data used:	1) Y
	2) V89_04, ~~V89_03~~ V89_13 corrected on 17Jun14
For manual calculation	1) Not applicable

1.5 Signature

[redacted]

Two-group t-tests (TOST) of equivalence in means (equal n's)

	1	2	3	4	5	6	7	8	9	10	11	12
Test significance levels, α (one-sided)	0,025	0,025	0,025	0,025	0,025	0,025	0,025	0,025	0,025	0,025	0,025	0,025
Lower equivalence limit for $\mu_T - \mu_S$, Δ_L	-0,176	-0,176	-0,176	-0,176	-0,176	-0,176	-0,176	-0,176	-0,176	-0,176	-0,176	-0,176
Upper equivalence limit for $\mu_T - \mu_S$, Δ_U	0,176	0,176	0,176	0,176	0,176	0,176	0,176	0,176	0,176	0,176	0,176	0,176
Expected difference, $\mu_T - \mu_S$	0,000	0,000	0,000	0,000	0,000	0,000	0,041	0,041	0,041	0,041	0,041	0,041
Common standard deviation, σ	0,650	0,700	0,750	0,800	0,850	0,880	0,650	0,700	0,750	0,800	0,850	0,880
Power (%)	95	95	95	95	95	95	95	95	95	95	95	95
n per group	421	488	560	636	718	770	604	700	804	914	1032	1106

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Documentation of Sample Size Calculation V89_18

One group χ^2 test that proportion equals user specified value (normal approximation)

	1	2	3	4	5
Test significance level, α	0,050		0,050		
1 or 2 sided test?	2		2		
Null hypothesis proportion, π_0	0,700		0,600		
Alternative proportion, π_A	0,860		0,810		
Power (%)	99		99		
n	1077		1077		

20.2 Review of Sample Size Calculation Final Dated 17Jun2014

Biostatistics & Statistical Reporting

Study number V89_18

Review of Sample Size Calculation

Author(s):	[redacted]
Document type:	Sample Size Review Documentation
Document status:	Final
Release date:	14-JUN-2014
Number of pages:	4

1 Sample Size Section for Protocol

The following sample size calculation has been subject to a review:

Data observed in previous NVD studies in similar sets (V89_04 in subjects 18-64 years of age; V89_13 in subjects > 65 years of age) showed a variability of the post vaccination titers ranging from 0.75 to 0.88 (in the log scale). With the proposed sample size, assuming a standard deviation of 0.85 for the log10 antibody titers (for each vaccine lot), approximate pairwise equivalence of factor 1 and independency, a single equivalence test based on 718 subjects per lot group has a power of 95%. The resulting overall power is approximately 86%, because the total number of comparisons is three. To account for dropouts (approximately 10%), a total of n=798 per lot should be recruited.

Data observed in previous NVD studies in similar populations (V89_04 in subjects 18- <65 years of age; V89_13 in subjects > 65 years of age) showed a proportion of 86% of subjects aged 18 to <65 years and a proportion of 81% of subjects aged 65 years and older achieving an HI antibody titer of ≥ 1:40. With a sample size of 1077 evaluable subjects from the pooled lots there is an overall power of approximately 98% to achieve the CBER criteria (HI antibody titer ≥ 1:40 for at least 70% of the subjects aged 18 to <65 years, and for at least 60% of the subjects aged 65 years and older). To account for dropouts (approximately 10%), a minimum sample size of n=1197 for all lots combined are needed.

2 Information Used for Reproduction of Sample Size

Co-primary 1

Sample size package used	SAS proc power Screenshots added below See also SAS program V89_18 - Equivalence.SAS located in folder Home / analysis / v89 / v89_18 / sample_size / valid / sas
Method used, with reference (if deemed necessary)	Two-groups t-test of equivalence of means (equal n's)
For manual calculation	Not applicable

Lower Bound	Upper Bound	Standard Deviation	Nominal Power	Alpha	Actual Power	GMT ratio 1.0	GMT ratio 1.1
-0.176	0.176	0.65	0.95	0.025	0.950	421	604
-0.176	0.176	0.70	0.95	0.025	0.950	488	700
-0.176	0.176	0.75	0.95	0.025	0.950	560	804
-0.176	0.176	0.80	0.95	0.025	0.950	636	914
-0.176	0.176	0.85	0.95	0.025	0.950	718	1032
-0.176	0.176	0.88	0.95	0.025	0.950	770	1106

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Documentation of Sample Size Calculation Review Study no

Co-primary 2

	Modify / delete whatever is applicable
Sample size package used	SAS proc power Screenshots added below See also SAS program V89_18 - Sufficiency.SAS located in folder Home / analysis / v89 / v89_18 / sample_size / valid / sas
Method used, with reference (if deemed necessary)	Binary data – One-sample design – Superiority
For manual calculation	Not applicable

Fixed Scenario Elements	
Method	Exact
Number of Sides	1
Null Proportion	0.7
Binomial Proportion	0.86
Total Sample Size	1077
Nominal Alpha	0.05

Computed Power			
Lower Crit Val	*Upper Crit Val*	*Actual Alpha*	*Power*
.	780	0.0435	>.999

Fixed Scenario Elements	
Method	Exact
Number of Sides	1
Null Proportion	0.6
Binomial Proportion	0.81
Total Sample Size	1077
Nominal Alpha	0.05

Computed Power			
Lower Crit Val	*Upper Crit Val*	*Actual Alpha*	*Power*
.	674	0.0444	>.999

3. Outcome of Review

☒ Sample size was verified.

o Sample size was not verified. Describe inconsistencies:

[redacted]

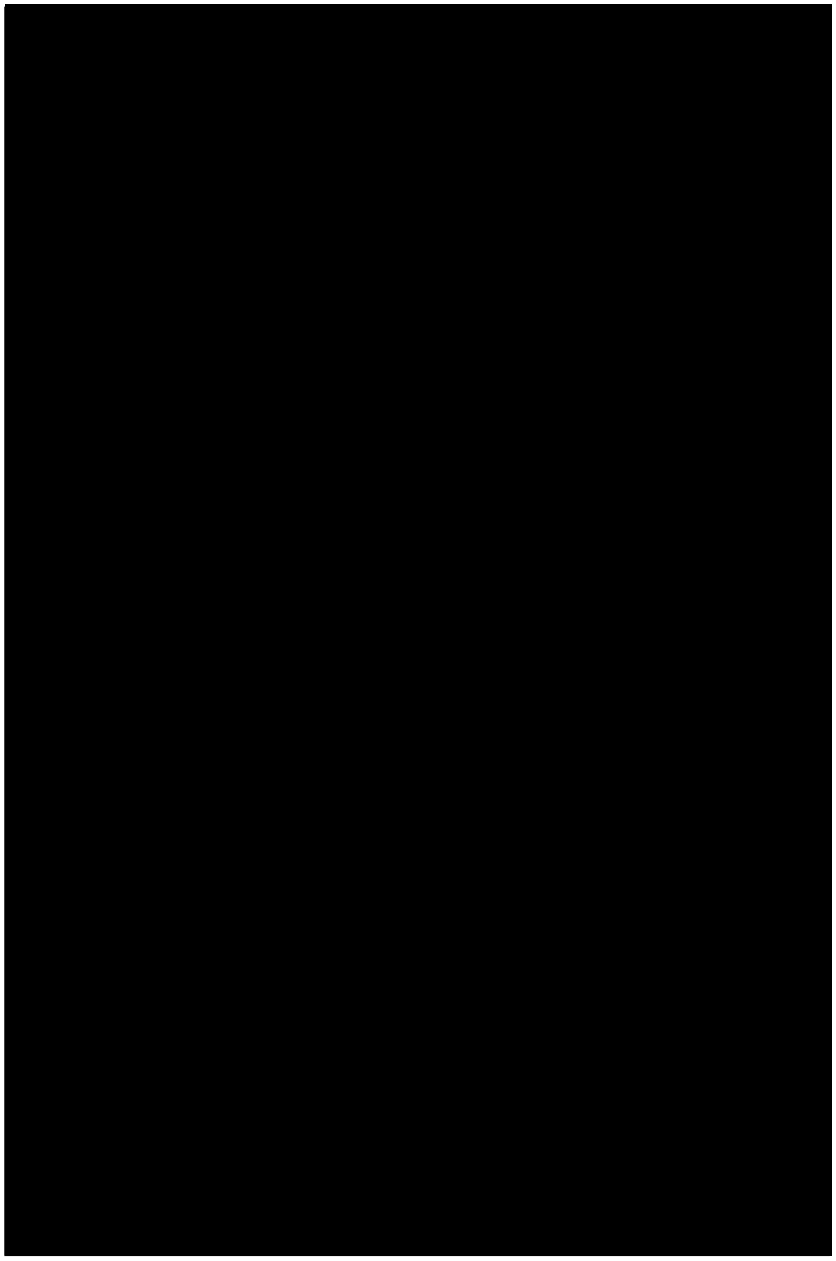
In case sample size was not verified

o Sample size discrepancies were resolved. A new Sample Size Calculation Memo (version 2) was issued.

C-BS Signature *Date (DD MMM YYYY)*

SPONSOR SIGNATURE PAGE

Approved by:



PAREXEL SIGNATURE PAGE

Authored by:

[redacted]

Reviewed by: