Statistical Analysis Plan for Interim Analysis of Phase 2 Cohort

Protocol No.: IVACFLU-A/H5N1 PHASE 2/3

Protocol Title:

A Phase 2/3 Double Blinded, Randomized, Placebo-Controlled Study In Healthy Adult Volunteers In Vietnam To Examine The Safety And Immunogenicity Of An Inactivated A/H5N1 Influenza Vaccine (IVACFLU-A/H5N1) Produced By IVAC

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PREFACE

The present analysis plan provides additional details regarding the planned interim analysis based on IVACFLU-A/H5N1 PHASE 2/3 study protocol version 1.0 (21Aug, 2015). The full SAP for the entire study will be developed separately. This document also expands and complements the study protocol providing further details regarding the preparation, contents, and presentation of statistical results along with the recommended Phase 3 dose to facilitate the decision whether to progress to the phase 3 component. The analysis plan will be reviewed and approved prior to the data freeze for the interim analysis. Any differences from the analysis plan summary in the protocol are also described in this plan.

This IVACFLU-A/H5N1 Phase 2/3 is a double-blind, randomized, placebo-controlled trial to test the safety and immunogenicity of two doses given 21 days apart of the IVAC A/H5N1 vaccine. In the Phase 2 study which will be conducted at one site (Khanah Hoa) , 300 male and female healthy adult subjects from 18 through 60 years of age will be randomized to receive 15 mcg of vaccine (n=100), 30 mcg of vaccine (n=100), or saline placebo (n=100). Safety and immunogenicity will be assessed in all 300 subjects. All subjects in Phase 2 and Phase 3 will receive two injections of A/H5N1 vaccine or placebo, 21 days apart.

Although full evaluation of the safety will continue through Day 91, this interim analysis will evaluate the complete safety and immunogenicity data collected through Day 43 of Phase 2 cohort to determine whether to proceed to Phase 3 and what dose to select. Vaccination of subjects in Phase 3 component will not begin until the DSMB has reviewed safety and immunogenicity data through Day 43 of phase 2 for all participants and concurs with the progression. . The conduct of Phase 3 will be dependent on a post dose two HAI titer of ≥1:40 in 60% or more vaccine recipients in at least one of the two vaccine groups in Phase 2 cohort.

I. OBJECTIVES

The study objectives and endpoints for Phase 2 as described in the protocol are as follows:

Primary Objective	**Endpoint**
Safety: To evaluate the safety and tolerability of two injections given 21 days apart of A/H5N1 vaccine at two dose levels (15 and 30 mcg).	The safety profile of IVACFLU-A/H5N1 will be evaluated by the proportion of subjects experiencing AEs, related or not related, of the following five categories for all subjects and by age group (18-40 years of age and 41-60 years age): • Number and percentage of subjects with solicited local and systemic adverse events within 30 minutes after each vaccination. • Number and percentages of subjects with solicited local adverse events (redness, swelling, pain, induration, and tenderness) over a 7-day period (Days 1-7; Days 22-28) post each vaccination. • Number and percentages of subjects with solicited systemic/general adverse events (fever, fatigue/malaise, muscle aches (generalized), joint aches, chills, nausea, vomiting, and headache) over a 7-day period (Days 1-7; Days 22-28) post each vaccination.

	• Number and percentages of subjects with unsolicited adverse events for 21 days post each vaccination. • All serious adverse events (SAEs) occurring over the entire study period. (Days 1-91).
Immunogenicity: To evaluate the immunogenicity of two different doses (15 mcg and 30 mcg) of A/H5N1.	**Primary**: Percentage of subjects achieving an HAI titer ≥1:40 after the second vaccination on Day 43 **Secondary**: • Percentage of subjects with a serum HAI titer ≥ 1:40 at Day 22. • Percentage of subjects demonstrating an HAI seroresponse of at least a four-fold increase in postvaccination titer on Day 22 and Day 43. • Percentage of subjects with at least a four-fold rise in titer at Day 22 and Day 43, as determined by microneutralization (MN). • Geometric Mean Titer (GMT) of Day 22 and Day 43 as determined by the HAI and MN tests. • Geometric Mean Titer Ratio (GMTR) of Day 22/Day 1 and Day 43/Day 1 as determined by the HAI and MN tests.

II. ANALYSIS POPULATIONS

The need for different analysis populations depends on how well the protocol is followed by study personnel and participants as well as the objectives of specific analyses. The final statistical report will include an accounting of all persons screened, including the number enrolled, the number followed during the study, and other key study status indicators. The reason for excluding any data from any analysis population will be documented.

A. Enrolled Population

All screened subjects who provide informed consent, regardless of the subject's randomization and treatment status in the trial.

B. Full Analysis (FA) Population

All subjects in the enrolled population who were randomized, received a study vaccination. This population will serve as the primary analysis population for all safety objectives. The analysis based on this population may serve as the supportive results for all immunogenicity objectives. Subjects who withdraw or are terminated from the study will be included in the FA data set until the time of withdrawal or termination. Subjects will be analyzed as received; that is, allocation errors, if any, are taken into account.

C. Per Protocol (PP) Population

All subjects in the Full Analysis population who have valid baseline (Day 1)[1]and a post-vaccination immunogenicity measure with no major protocol violations that are determined to potentially interfere with the immunogenicity assessment of the study vaccine. This population will serve as the primary analysis population for all immunogenicity objectives.

The criteria for exclusion of subjects from the Full Analysis Population and Per Protocol Population will be established by the study statistician and the study medical officer before breaking the blind and will be based on the blinded review of protocol violations.

III. MISSING DATA

In the event that missing dates or times are needed to compute durations of outcomes, the following rules will be applied:

- If the month and year are known, but the date is missing, the 15th will be used for any calculations of relative time (e.g., UNMAY2012 will become 15MAY2012).

- If only the year is known, but the month and date are missing, June 15th of the known year will be substituted for any calculations of relative time (e.g., UNUNK2011 will become 15JUN2011).

- If the minutes of start or stop times are missing, time will be assumed to be on the hour for a 24 hour clock (e.g., 11:UN, is assumed to be 11:00 AM).

If some safety data is available for the subject in safety population but respective data is missing then subject will be included in the analysis and data will be treated as follows:

1. For solicited AEs, if the severity for all 7 days are missing or the diary card was not completed or was marked as 'unknown' then the aggregated value will be set to missing and the subject will be excluded from the analysis. If the intensities are missing for only some of the days then the missing intensities will be evaluated as the maximum of the previous and next non-missing value for calculation of the aggregated value. For example if fever intensity is recorded as 1, (blank field), 2, 0, 0, for day 0 to day 4, then the missing day 2 intensity is imputed to be 2. Severity is missing for any AE then it will be considered as AE of maximum severity (Grade 3) "Severe" unless it's captured as SAE. For SAE missing severity will be considered as maximum severity (Grade 4) "Life Threatening"
2. If "Relationship" is missing then it will be considered as "Related" to vaccine administered i.e. study vaccine/ placebo.
3. If for Start date - day of event / condition is missing for any adverse event then it will be imputed as the date of last dose of study vaccine / placebo.
4. If for Start date - day and month of any adverse event is missing then it will be imputed as the date of first dose of study vaccine / placebo.
5. If Stop date of any adverse event is missing then it will be treated as missing.

[1] The protocol does not state this requirement.

All immunogenicity assays reported below the limit of quantification (LLOQ, e.g. <1:10) will be assigned a value of one half the lower limit of quantification (LLOQ) or threshold value (e.g. 0.5 for the given example).

IV. UNBLIDING STUDY CODE FOR INTERIM DATA ANALYSES

The planned interim study report for the Phase 2 cohort described in this analysis plan (SAP) will initially be prepared by blinded study product group. After all analysis programs and output tables for the report are verified, the reports will be regenerated by re-running all analysis programs with the unblinded study product code given by the study randomisation statistician. The FHI360 randomisation statistician will verify with IVAC that no errors were made in study product assignment and dispensing of vaccines. All study site and FHI360 personnel involved in the study conduct will remain blinded at subject level other than the top line results until the completion of the Phase 2 cohort. The study subjects will remain blinded until the completion of the Phase 2 cohort. Selected members of PATH and the sponsor, IVAC, will be unblinded at group level to deliberate the dose selection and the preparation of the interim study report.

V. MULTIPLICITY CONSIDERATION

In general, since the purpose of this interim assessment is to select the dose for the Phase 3 component and the final confirmatory analysis will be solely based on the data generated for the Phase 3 component only, there is no need to make an adjustment to the Type I error rate between the Phase 2 and Phase 3 components of the study.

Specifically, the analysis of immunogenicity data has a single primary endpoint and it maintains the experiment-wise Type I error rate at 2-sided of 0.05. No multiplicity adjustment to the error rate, alpha, will be made for secondary immunogenicity endpoints.

In addition, the analysis of safety data is considered to be an initial screening step in the sense that treatment comparisons will be carried out for the purposes of identifying safety concerns for further clinical evaluation. No formal hypothesis testing with multiplicity adjustment will be performed. Furthermore, no statistical testing will be performed for unsolicited AEs including SAEs.

VI. DESCRIPTIVE ANALYSIS

A. Study Participant Disposition

A flowchart or table showing the number screened, enrolled, randomized, vaccinated, withdrawal and its reasons, and numbers in the Full Analysis and Per Protocol Populations with protocol deviations reported on the Protocol Deviation/Violation (PDV) CRF or detectable in the data base will be produced.

B. Descriptive Statistics

Descriptive Statistics will be presented in summary tables by each group, each time visit and overall. Categorical variables will be summarized by frequencies and percentages. Continuous variables will be summarized by means, standard deviations, medians, interquartile ranges, minima and maxima.

No participant-specific listings will be presented in this interim analysis.

C. Baseline Data

Following information on enrollment will be summarized by study group for FA Population; baseline demographics, medical history, serology, vital signs, chemistries and hematology, pregnancy test and physical examination. Baseline measures are those obtained closest to but preceding randomization and vaccine #1. No inferential statistics (e.g. p-values, confidence intervals) will be computed comparing treatment groups on baseline variables.

D. Laboratory Data

The immunogenicity measures will be expressed by Hemagglutination Inhibition (HAI) titers and titers evaluated by Micro neutralization tests (MN), and also expressed as geometric mean titer (GMT) of the antibody response. Because titers are often approximately lognormally distributed, they are customarily analyzed on a logarithmic (log) scale; a difference in arithmetic means on a log scale becomes a ratio of geometric means when the results of analysis are converted back to the original data scale.[2]

It is possible that samples fall below the cut-off or LLQ of some antigens. Values below the cut-off of the assay will be given an arbitrary value of half the LLQ for the purpose of the calculation. Similarly, for lab value above MDL an arbitrary value of 2-fold the MDL will be given for the purpose of the calculation. The final details about the cut-off values will be provided by the study lab.

For each group and at each time-point (Day 1, Day 22 and Day 43) that a blood-sample result was available following descriptive analyses were performed:

- Frequencies and proportions of available specimens, valid immune responses and measurements below/above certain cut-off will be tabulated.
- GMTs and their 95% confidence intervals (CIs) will be calculated by taking the anti-log of the mean of the log concentration or titer transformations.
- Distributions of antibody titers (log scale) will be displayed using reverse cumulative distribution curves.
- The reasons for excluding from the immunogenicity analysis will be listed.

VII. ANALYSIS OF IMMUNOGENICITY OBJECTIVES

All immunogenicity endpoint analyses and summaries will be performed on a per-protocol basis for all subjects and by age group (18-40 years of age and 41-60 years age). Supportive analysis will also be conducted on participants in the FA population who received at least one dose of study vaccines if the difference in the number of subjects between the FA and PP populations exceeds 5%.

Objective: To evaluate the immunogenicity of two different doses (15 mcg and 30 mcg) of A/H5N1.

[2] Horne AD, Lachenbruch PA, Getson PR, and Hsu HS Analysis of Studies to Evaluate Immune Response to Combination Vaccines. Clinical Infectious Diseases 2001; 33(Suppl 4):S306–11

There are two types of immunogenicity endpoints, percentages of subjects with immune response and Geometric Mean Titers (GMT), will be used to evaluate this study objective.

Percentages of subjects with immune response:

- Percentage of subjects achieving an HAI titer ≥1:40[3] on Day 43. (primary endpoint)
- Percentage of subjects with a serum HAI titer ≥ 1:40 at Day 22.
- Percentage of subjects demonstrating an HAI seroresponse of at least a four-fold increase in postvaccination titer on Day 22 and Day 43.
- Percentage of subjects with at least a four-fold rise in titer at Day 22 and Day 43, as determined by microneutralization (MN).

For each percentage of subjects with defined immune response ednpoints, the percentage and its corresponding 2-sided exact (Clopper-Pearson) binomial 95% confidence interval (95% CI) around the percentage will be computed for each treatment group.

In addition, to compare HAI and MN test seroresponse, individually, between the two dose groups, two-sided 95% CIs for the difference in the percentages of participants demonstrating seroresponse between the two dose groups will be provided for each time-point (Day 22 and Day 43) based on the Newcombe hybrid score (Newcombe, 1998)[4].

Geometric Mean Titers (GMTs):

- Geometric Mean Titer (GMT) of Day 22 and Day 43 as determined by the HAI and MN tests.
- Geometric Mean Fold Rise (GMFR): Geometric Mean of Titer Ratio of Day 22/Day 1 and Day 43/Day 1 as determined by the HAI and MN tests.

GMT along with the corresponding two-sided 95% CIs, by exponentiating the corresponding log-transformed means and their 95% CIs, will be summarized by treatment group, the HAI and MN tests, and each time-point (Day1, Day 22 and Day 43). The same approach will be used to summarize GMFR separately by treatment group, Day 22/Day 1 and Day 43/Day1, and the HAI and MN tests.

Furthermore, to compare two dose groups based on GMT endpoints, two-sided 95% CIs for the ratios post-vaccination GMT between two dose groups will be constructed using a log-normal distribution, separately for Day 22 and Day 43 and the HAI and MN tests. The log values will be used to construct a 95% CI using t-distribution for the mean difference between the two dose groups. Then the mean difference and the corresponding 95% CI limits will be exponentiated to obtain the GMT ratio and the corresponding CI. The same approach will be used to compare two dose groups based on GMFRs

[3] Note that this endpoint is expected to be equivalent to another commonly used endpoint of seroconversion as defined as a post-vaccination titer ≥1:40 if baseline titer <1:10 OR significant increase in antibody titer post vaccination (at least a four-fold increase from baseline titer) if baseline titer is ≥1:10, since all subjects are expected to have baseline titer of < 1:10. If at least one subject has baseline titer ≥1;10, an additional endpoint of seroconversion as defined above will also be analyzed

[4] Newcombe, R. G. (1998), "Interval Estimation for the Difference Between Independent Proportions: Comparison of Eleven Methods," Statistics in Medicine, 17, 873–890.

separately by Day 22/Day 1, Day 43/Day1, and the HAI and MN tests. No comparisons will be made between the IVACFLU-A/H5N1 dose groups and the placebo group.

VIII. ANALYSIS OF SAFETY OBJECTIVES

The safety profile will be parameterized as the frequency and proportion of specific symptoms/problems in each group falling into one of the following five categories for all subjects and by age group (18-40 years of age and 41-60 years age).

- **Immediate reactions** (solicited local and systemic adverse events), occurring in the first 30 minutes post vaccination on Day 1 and Day 22, as observed by study staff. The reaction will include
    - Local signs/symptoms: redness, swelling, hardness, pain, or tenderness of the injected site of upper arms;
    - Systemic signs/symptoms: headache, fever, fatigue/malaise, muscle aches, joint aches, nausea, vomiting, and chills.

- **Solicited adverse local reactions** occur over a 7-day period (Days 1-7; Days 22-28) post each vaccination, identified or observed by study staff during home visits and/or reported by parent at any time through Day 7. Specifically, the following local signs and symptoms at site of injection will be assessed and documented as solicited adverse reactions:
    - Redness
    - Swelling
    - Induration
    - Pain
    - Tenderness

- **Solicited adverse systemic reactions** occur over a 7-day period (Days 1-7; Days 22-28) post each vaccination, identified or observed by study staff during home visits and/or reported by parent at any time through Day 7. Specifically, the following systemic reactions will be assessed and documented as solicited adverse reactions:
    - Fever/body temperature (and body location of measurement)
    - Fatigue/malaise
    - Generalized muscle aches
    - Joint aches/pains
    - Chills
    - Nausea
    - Vomiting
    - Headache Body temperature

  If a solicited sign or symptom has started during the 7 days post vaccination and continues beyond the 7 days, it will continue to be reported as a reactogenicity symptom. Any solicited sign or symptom starting after 7 days post vaccination will be recorded as an "unsolicited AE". Adverse Reactions will be graded (i.e., Grade 1 to 4) and subcategorized as those deemed related to vaccination or not by a study clinician.

- **Unsolicited adverse events** are AEs that are not solicited and occurs any time during 21 days post each vaccination**.**

- **Serious adverse events** (SAEs) occur from vaccination through 3 months (Days 1-91) post-vaccination, identified or observed by study staff and/or reported by parent at any time. SAEs will be graded for severity and subcategorized as those deemed related to vaccination or not by a study clinician. All SAEs will be listed.

In general, adverse events (AEs) will be summarized by MedDRA System/Organ Class (SOC) and adverse event type (MedDRA Preferred Term), using the MedDRA dictionary in production at FHI 360 at the time the database is developed. As needed, this AE summary will be repeated for (a) AEs considered related to study product and (b) serious AEs (SAEs). In Addition, concomitant medications will be coded and listed.

All AE summaries will report the number of persons with at least one episode of the given AE type/SOC, as well as the number of episodes, separately for "any local AE" or "any systemic AE". In calculating percentages, each person will contribute only once to a given AE type/SOC no matter the number of episodes experienced. Percentages of subjects experiencing each reaction or event, or at least one reaction or event, will be calculated along with two-sided exact (Clopper-Pearson) binomial 95% CIs.

For the solicited local and systemic/general adverse events, Fisher's exact test for two proportions or the Cochran-Mantel-Haenszel test for severity grade categories at 2-sided 0.05 alpha will be used to compare the treatment groups.

In addition, subject listings of adverse events will include details such as the following: MedDRA codes, study day of onset, seriousness, duration, action/treatment, outcome, severity, relatedness, and current status.

Vital Signs and Physical Examination Data: Physical examination, vitals, and laboratory data if available will be summarized for post-vaccination measurements, separately by visit or time point of observation. Baseline vital signs and physical exam data will be presented in comparison with the post-vaccination results; changes from baseline to 7 days post-vaccinations will be summarized.

IX. REPORTS FOR DSMB INITIAL COHORT REVIEW

A statistical report and the recommended dose for the Phase 3 component will be prepared for the DSMB initial cohort review meeting. The report will include figures, tables for analyses results from safety and immunogenicity data, and listings broken down by treatment group as attached in Appendix.

Proposed Dose Selection Criteria for Phase 3 Component:

The higher vaccine dose tested (30 mcg) will be selected if it induces higher immune responses than the lower dose as described in the table below. In addition other factors such as secondary immunogenicity endpoints, safety data, and manufacturing costs will also be considered. The recommended dose for the Phase 3 component will be chosen by the IVAC and PATH stake holders prior to the DSMB initial cohort review meeting.

Dose	Percentage of subjects with HAI ≥ 40 after two vaccinations			
Low dose (15 mcg)	< 60%	60-64%	65-74%	≥ 75%
High dose (30 mcg)	≥ 60%	> 15 mcg dose	> 13% higher	Any
Dose selection	30 mcg	30 mcg	15 mcg, if Δ <13%	15 mcg
			30 mcg if Δ ≥13%	

Note Δ represent a difference in HAI in the high and low dose group

Final Statistical Analysis Plan for Phase 3 Cohort

Protocol No.: IVACFLU-A/H5N1 -0203

Version Number: 3

Date: 24 Oct 2016

Protocol Title:

A Phase 2/3 Double Blinded, Randomized, Placebo-Controlled Study In Healthy Adult Volunteers In Vietnam To Examine The Safety And Immunogenicity Of An Inactivated A/H5N1 Influenza Vaccine (IVACFLU-A/H5N1) Produced By IVAC

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PREFACE

The present analysis plan provides additional details regarding the planned final analysis of Phase 2/3 cohort based on IVACFLU-A/H5N1 PHASE 2/3 study protocol version 3.0 (24Oct, 2016). It will be reviewed and approved by PATH, IVAC and WHO at least two weeks before final data freeze. Any differences from the analysis plan summary in the protocol are also described in this plan.

This IVACFLU-A/H5N1 Phase 2/3 is a double-blind, randomized, placebo-controlled trial to test the safety and immunogenicity of two doses given 21 days apart of the IVAC A/H5N1 vaccine. Subjects will be male and female healthy adults from 18 through 60 years of age. In the Phase 2 study which was a dose-selection study and was conducted at one site (Khanh Hoa), 300 male and female healthy adult subjects from 18 through 60 years of age were randomized to receive 15 mcg of vaccine (n=100), 30 mcg of vaccine (n=100), or saline placebo (n=100). The details of the interim analysis plan for the Phase 2 study had been prepared separately.

The Phase 3 is a confirmatory study that will expand the safety and immunogenicity in a larger number of subjects to meet product registration in Vietnam. In Phase 3, subjects will be randomized to receive the selected vaccine 15 mcg dose (n=500) or saline placebo (n=100) under double blind. Safety will be assessed in all subjects and immunogenicity will be measured by Hemagglutination Inhibition test (HAI) in a subset of approximately 270 subjects receiving the IVACFLU-A/H5N1 (n=225 vaccinees) and placebo (N=45) from all subjects enrolled at one of 2 study sites (Hai Phong) in Phase 3.

This final analysis plan will mainly focus on the analyses of Phase 3 cohort with additional analyses combining subjects from both phases if needed.

I. OBJECTIVES

The study objectives and endpoints for Phase 3 as described in the protocol are as follows:

Primary Objective	**Endpoint**
Safety: To evaluate the safety and tolerability of two injections given 21 days apart of A/H5N1 vaccine at the dose level of 15 mcg.	The safety profile of IVACFLU-A/H5N1 will be evaluated by the proportion of subjects experiencing AEs, related or not related, of the following five categories for all subjects and by age group (18-40 years of age and 41-60 years age): • Number and percentage of subjects with solicited local and systemic adverse events within 30 minutes after each vaccination. • Number and percentages of subjects with solicited local adverse events (redness, swelling, pain, induration, and tenderness) over a 7-day period (Days 1-7; Days 22-28) post each vaccination. • Number and percentages of subjects with solicited systemic/general adverse events (fever, fatigue/malaise, muscle aches (generalized), joint aches, chills, nausea, vomiting, and headache) over a 7-day period (Days 1-7; Days 22-28) post each vaccination.

	• Number and percentages of subjects with unsolicited adverse events for 21 days post each vaccination. • All serious adverse events (SAEs) occurring over the entire study period. (Days 1-91).
Immunogenicity: To evaluate the immunogenicity of the A/H5N1 influenza vaccine as determined by serum HAI titer ≥ 1:40 at Day 43 in a sample of approximately 270 subjects receiving the IVACFLUA/ H5N1 (vaccinees) and placebo.	**Primary**: Percentage of subjects achieving an HAI titer ≥1:40 after the second vaccination on Day 43. **Secondary**: • The percentage of subjects achieving a seroresponse (at least a four-fold increase in post-vaccination titer) on Day 43 as determined by HAI. • Geometric Mean Titer (GMT) of Day 43 as determined by HAI. • Geometric Mean Titer Ratio (GMTR) of Day 43/Day 1 as determined by HAI.

II. ANALYSIS POPULATIONS

The need for different analysis populations depends on how well the protocol is followed by study personnel and participants as well as the objectives of specific analyses. The final statistical report will include an accounting of all persons screened, including the number enrolled, the number followed during the study, and other key study status indicators. The reason for excluding any data from any analysis population will be documented.

A. Enrolled Population

All screened subjects who provide informed consent, regardless of the subject's randomization and treatment status in the trial.

B. Full Analysis (FA) Population

All subjects in the enrolled population who were randomized, received a study vaccination. This population will serve as the primary analysis population for all safety objectives. The immunogenicity analysis based on this population will serve as the supportive results for all immunogenicity objectives. . Subjects who withdraw or are terminated from the study will be included in the FA data set until the time of withdrawal or termination. Subjects will be analyzed as received; that is, allocation errors, if any, are taken into account.

C. Per Protocol (PP) Population

All subjects in the Full Analysis population who have valid baseline (Day 1)[1]and a post-vaccination immunogenicity measure with no major protocol violations that are determined to potentially interfere

[1] The protocol does not state this requirement.

with the immunogenicity assessment of the study vaccine (e.g., missed the 2nd vaccination). This population will serve as the primary analysis population for all immunogenicity objectives. [2]

The criteria for exclusion of subjects from the Full Analysis Population and Per Protocol Population will be established by the study statistician and the study medical officer before breaking the blind and will be based on the blinded review of protocol violations.

III. MISSING DATA

In the event that missing visit dates or study contact dates are needed to be imputed for couples of study forms, the following rules will be applied:

- The month and year are known, but the date was missing, the 15th was used for any calculations of relative time (e.g., UNMAY2016 will become 15MAY2016).

For missing safety related data, the following rules will be applied:

- For solicited AEs, if the severity for all 7 days are missing or the diary card was not completed or was marked as 'unknown' then the aggregated value will be set to missing and the subject will be excluded from the analysis. If the intensities are missing for only some of the days then the missing intensities will be evaluated as the maximum of the previous and next non-missing value for calculation of the aggregated value. For example if fever intensity is recorded as 1, (blank field), 2, 0, 0, for day 0 to day 4, then the missing day 2 intensity is imputed to be 2. Severity is missing for any AE then it will be considered as AE of maximum severity (Grade 3) "Severe" unless it's captured as SAE. For SAE missing severity will be considered as maximum severity (Grade 4) "Life Threatening"
- If "Relationship" is missing then it will be considered as "Related" to vaccine administered i.e. study vaccine/ placebo.
- If for Start date - day of event / condition is missing for any adverse event then it will be imputed as the date of last dose of study vaccine / placebo.
- If for Start date - day and month of any adverse event is missing then it will be imputed as the date of first dose of study vaccine / placebo.
- If Stop date of any adverse event is missing then it will be treated as missing.

All immunogenicity assays reported below the limit of quantification (LLOQ, e.g. <1:10) will be assigned a value by the study lab. No missing data will be imputed.

[2] This definition is similar to the criteria: 1) the completion of a certain pre-specified minimal exposure to the treatment regimen; 2) the availability of measurements of the primary variable(s); 3) the absence of any major protocol violations including the violation of entry criteria; that given by ICH guideline E9: statistical principles for clinical trials: International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; 1998. Available from: http://www.ich.org/products/guidelines/efficacy/article/efficacy-guidelines.html.

IV. UNBLIDING STUDY CODE FOR FINAL DATA ANALYSES

Before unblinding the study data, FHI360 statisticians will provide blinded data to the study statistician and the study medical officer to establish the criteria for exclusion of subjects from the Full Analysis Population and Per Protocol Population, and to review protocol violations. The final data analysis will initially be prepared with blinded study product group. After all analysis programs and output tables for the final analyses are verified, all analysis programs will be re-ran with the unblinded study product code given by the study randomisation statistician. In addition, the FHI360 randomisation statistician will verify with IVAC that no errors were made in study product assignment and dispensing of vaccines.

V. MULTIPLICITY CONSIDERATION

In general, since the purpose of the Phase 2 assessment is to select the dose for the Phase 3 component and the final confirmatory analysis will be solely based on the data generated for the Phase 3 component, there is no need to make an adjustment to the Type I error rate between the Phase 2 and Phase 3 components of the study.

Specifically, the analysis of immunogenicity data has a single primary endpoint and it maintains the experiment-wise Type I error rate at 2-sided of 0.05. No multiplicity adjustment to the error rate, alpha, will be made for secondary immunogenicity endpoints.

In addition, no formal hypothesis testing will be conducted for safety data analyses, therefore, no multiplicity adjustment will be performed.

VI. DESCRIPTIVE ANALYSIS

A. Study Participant Disposition

A flowchart or table showing the number screened, enrolled, randomized, vaccinated, withdrawal and its reasons, and numbers in the Full Analysis and Per Protocol Populations with protocol deviations reported on the Protocol Deviation/Violation (PDV) CRF will be produced.

B. Descriptive Statistics

Descriptive statistics will be presented in summary tables by each group, each time visit and overall. Categorical variables will be summarized by frequencies and percentages. Continuous variables will be summarized by means, standard deviations, medians, interquartile ranges, minima and maxima.

C. Baseline Data

Following information on enrollment will be summarized by study group for FA Population; baseline demographics, medical history, vital signs, pregnancy test and general physical examination. Baseline measures are those obtained closest to but preceding randomization and vaccine #1. No inferential statistics (e.g. p-values, confidence intervals) will be computed comparing treatment groups on baseline variables.

D. Laboratory Data

The immunogenicity measures will be expressed by Hemagglutination Inhibition (HAI) titers and also expressed as geometric mean titer (GMT) of the antibody response. Because titers are often approximately lognormally distributed, they are customarily analyzed on a logarithmic (log) scale; a difference in arithmetic means on a log scale becomes a ratio of geometric means when the results of analysis are converted back to the original data scale.[3]

It is possible that samples fall below the cut-off or LLQ of some antigens. Values below the cut-off of the assay will be given an arbitrary value of half the LLQ for the purpose of the calculation. Similarly, for lab value above Method detection limit (MDL) an arbitrary value of 2-fold the MDL will be given for the purpose of the calculation. The final details about the cut-off values will be provided by the study lab.

For each group and at each time-point (Day 1 and Day 43) that a blood-sample result was available following descriptive analyses were performed:

- Frequencies and proportions of available specimens, valid immune responses and measurements below/above certain cut-off will be tabulated.
- GMTs and their 95% confidence intervals (CIs) will be calculated by taking the anti-log of the mean of the log concentration or titer transformations.
- Distributions of antibody titers (log scale) will be displayed using reverse cumulative distribution curves.
- The reasons for excluding from the immunogenicity analysis will be listed.

VII. ANALYSIS OF IMMUNOGENICITY OBJECTIVES

Objective: To evaluate the immunogenicity of the A/H5N1 influenza vaccine as determined by serum HAI titer ≥ 1:40 at Day 43 in a sample of approximately 270 subjects receiving the IVACFLUA/H5N1 (vaccinees) and placebo.

Analysis Population: All immunogenicity endpoint analyses and summaries will be performed on a per-protocol basis for all subjects and by age group (18-40 years of age and 41-60 years age). Supportive analysis will also be conducted on participants in the FA population who received at least one dose of study vaccines if the difference in the number of subjects between the FA and PP populations exceeds 5%.

Study Endpoints

Primary:

- Percentage of subjects achieving an HAI titer ≥1:40 after the second vaccination on Day 43

Secondary:

- The percentage of subjects achieving a seroresponse (at least a four-fold increase in post-vaccination titer) on Day 43 as determined by HAI
- Geometric Mean Titer (GMT) of Day 43 as determined by HAI.

[3] Horne AD, Lachenbruch PA, Getson PR, and Hsu HS Analysis of Studies to Evaluate Immune Response to Combination Vaccines. Clinical Infectious Diseases 2001; 33(Suppl 4):S306–11

- Geometric Mean Titer Ratio (GMTR) of Day 43/Day 1 as determined by HAI.

Analysis Methods

There are two types of immunogenicity measurements, percentages of subjects with immune response and Geometric Mean Titers (GMT), that will be used to evaluate this study objective.

Percentages of subjects with immune response:

- Percentage of subjects achieving an HAI titer ≥1:40[4] on Day 43.
- Percentage of subjects demonstrating an HAI seroresponse of at least a four-fold increase in postvaccination titer on Day 43.

For each percentage of subjects with the defined immune responses, the percentage and its corresponding 2-sided exact (Clopper-Pearson) binomial 95% confidence interval (95% CI) around the percentage will be computed for each treatment group and by age group.

Geometric Mean Titers (GMTs):

- Geometric Mean Titer (GMT) of Day 43 as determined by HAI
- Geometric Mean Fold Rise (GMFR): Geometric Mean of Titer Ratio of Day 43/Day 1 as determined by HAI.

GMT along with the corresponding two-sided 95% CIs, by exponentiating the corresponding log-transformed means and their 95% CIs, will be summarized by treatment group on Day1 and Day 43. The same approach will be used to summarize GMFR of Day 43/Day1 separately by treatment group and age group.

Furthermore, the analyses will be repeated by combining the immunogenicity measures of the selected dose group and the placebo group from both Phases 2 and 3 cohorts if needed. No comparisons will be made between the IVACFLU-A/H5N1 dose group and the placebo group.

VIII. ANALYSIS OF SAFETY OBJECTIVES

Objective: To evaluate the safety and tolerability of two injections given 21 days apart of A/H5N1 vaccine.

Analysis Population: The Full Analysis Population will be used for the safety objective analysis.

[4] Note that this endpoint is expected to be equivalent to another commonly used endpoint of seroconversion as defined as a post-vaccination titer ≥1:40 if baseline titer <1:10 OR significant increase in antibody titer post vaccination (at least a four-fold increase from baseline titer) if baseline titer is ≥1:10, since all subjects are expected to have baseline titer of < 1:10. If at least one subject has baseline titer ≥1:10, an additional endpoint of seroconversion as defined above will also be analyzed

Endpoints: The safety profile of IVACFLU-A/H5N1 will be evaluated by the number and the proportion of subjects experiencing AEs, related or not related, of the following categories for all subjects and by age group (18-40 years of age and 41-60 years age):

- **Immediate reactions** (solicited local and systemic adverse events), occurring in the first 30 minutes post vaccination on Day 1 and Day 22, as observed by study staff. The reaction will include
    - Local signs/symptoms: redness, swelling, hardness, pain, or tenderness of the injected site of upper arms;
    - Systemic signs/symptoms: headache, fever, fatigue/malaise, muscle aches, joint aches, nausea, vomiting, and chills.

- **Solicited adverse local reactions** occur over a 7-day period (Days 1-7; Days 22-28) post each vaccination, identified or observed by study staff during home visits and/or reported by parent at any time through Day 7. Specifically, the following local signs and symptoms at site of injection will be assessed and documented as solicited adverse reactions:
    - Redness
    - Swelling
    - Induration
    - Pain
    - Tenderness

- **Solicited adverse systemic reactions** occur over a 7-day period (Days 1-7; Days 22-28) post each vaccination, identified or observed by study staff during home visits and/or reported by parent at any time through Day 7. Specifically, the following systemic reactions will be assessed and documented as solicited adverse reactions:
    - Fever/body temperature (and body location of measurement)
    - Fatigue/malaise
    - Generalized muscle aches
    - Joint aches/pains
    - Chills
    - Nausea
    - Vomiting
    - Headache

  If a solicited sign or symptom has started during the 7 days post vaccination and continues beyond the 7 days, it will continue to be reported as a reactogenicity symptom. Any solicited sign or symptom starting after 7 days post vaccination will be recorded as an "unsolicited AE". Adverse reactions will be graded (i.e., Grade 1 to 4).

- **Unsolicited adverse events** are any AEs that occur any time after the vaccine/placebo is given (temporally related to study product), whether or not deemed "related" to the product, and are not solicited (specifically asked of the subject). Unsolicited AEs can be observed by study staff while the subject is at a clinic for a study visit or reported by the subject at any time, and subcategorized as those deemed related to vaccination or not by a study clinician.

- **Serious adverse events** (SAEs) occur from vaccination through 3 months (Days 1-91) post-vaccination, identified or observed by study staff and/or reported by parent at any time. SAEs will be graded for severity and subcategorized as those deemed related to vaccination or not by a study clinician. All SAEs will be listed.

- **Targeted physical examination and vitals data** will be summarized for post-vaccination measurements, separately by visit or time point of observation.

Analysis Methods

In general, adverse events (AEs) will be summarized by MedDRA System/Organ Class (SOC) and adverse event type (MedDRA Preferred Term), using the MedDRA dictionary in production at FHI 360 at the time the database is developed. As needed, this AE summary will be repeated for (a) AEs considered related to study product and (b) serious AEs (SAEs).

Unsolicited AE summaries will report the number of persons with at least one episode of the given AE type/SOC, as well as the number of episodes. In calculating percentages, each person will contribute only once to a given AE type/SOC no matter the number of episodes experienced. Percentages of subjects experiencing each reaction or event, or at least one reaction or event, will be calculated along with two-sided exact (Clopper-Pearson) binomial 95% CIs.

For the solicited local and systemic/general adverse events, Fisher's exact test for two proportions or the Cochran-Mantel-Haenszel test[5] for severity grade categories at 2-sided 0.05 alpha will be used to compare the treatment groups. No formal hypothesis testing with multiplicity adjustment will be performed. Furthermore, no statistical testing will be performed for unsolicited AEs including SAEs.

In addition, subject listings of adverse events will include details such as the following: MedDRA codes, study day of onset, seriousness, duration, action/treatment, outcome, severity, relatedness, and current status. Concomitant medications will also be coded and listed.

Furthermore, additional analyses will be repeated by combining safety outcomes of the selected dose group and placebo group from both Phases 2 and 3 cohorts if needed.

[5] The Cochran-Mantel-Haenszel test is better method for comparing severity grade categories than the Chi-squared test as proposed in the study protocol