# Statistical Analysis Plan

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| Protocol Title | Immunogenicity and safety of concurrent administration of live, attenuated SA 14-14-2 Japanese encephalitis vaccine and measles-mumps-rubella vaccine in infants 9-12 months of age in Philippines |
| Protocol Number | JEV06 Version 2.0, NCT02880865 |
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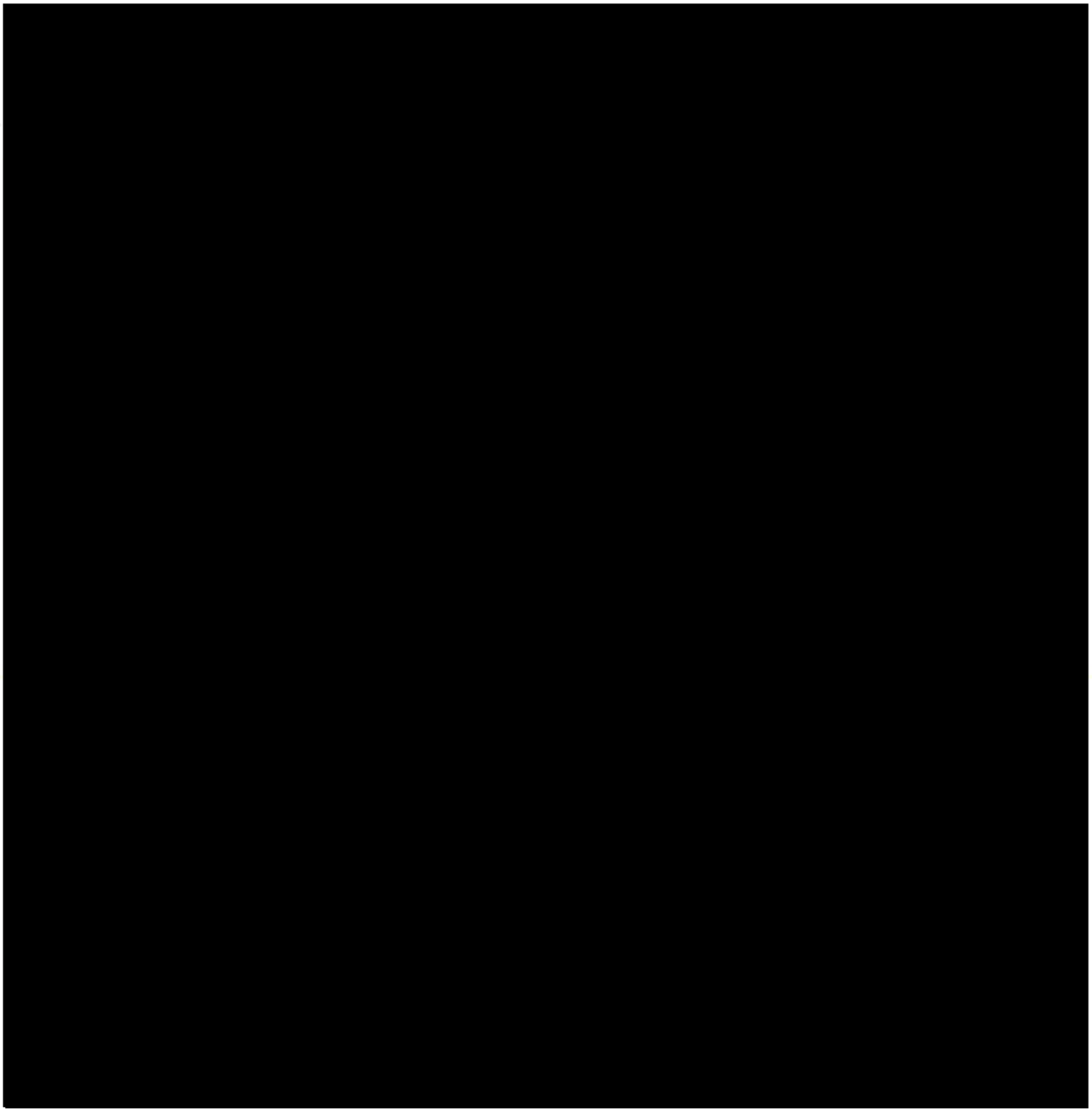
# Statistical Analysis Plan Signature Page



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## 1. List of Abbreviations

| | |
|---|---|
| AE | Adverse Event |
| ATC | Anatomical Therapeutic Chemical |
| CDIBP | Chengdu Institute of Biological Products |
| CD-JEV | Prequalified Live, Attenuated SA-14-4-2 Japanese Encephalitis Vaccine |
| CI | Confidence Interval |
| ELISA | Enzyme-linked Immunosorbent Assay |
| EPI | Expanded Programme on Immunization |
| GMC | Geometric Mean Concentration |
| GMT | Geometric Mean Titer |
| IgG | Immunoglobulin G |
| ITT | Intent to Treat |
| JE | Japanese Encephalitis |
| MCV | Measles Containing Vaccine |
| MMR | Measles, Mumps, Rubella Vaccine |
| PP | Per Protocol |
| PRNT | Plaque Reduction Neutralization Test |
| SAE | Serious Adverse Event |
| SAS | Safety Analysis Set |
| SD | Standard Deviation |
| WHO | World Health Organization |

# 2. Introduction

A live, attenuated SA 14-14-2 Japanese Encephalitis vaccine (CD-JEV) manufactured by the Chengdu Institute of Biological Products (CDIBP) has been prequalified by WHO. CD-JEV has been considered for a widespread use in protecting children from Japanese Encephalitis (JE) in JE-endemic countries. Based on the youngest age range evaluated in clinical development and to avoid the potential presence of maternal antibodies, the most logical time to administer CD-JEV is 9 to 12 months of age when measles containing vaccines (MCVs) are typically given in Asia. While an increasing number of JE-endemic countries are introducing or planning to introduce measles, mumps, rubella vaccine (MMR) into the Expanded Programme on Immunization (EPI), it is important to provide evidence that MMR can be introduced concurrently with other pediatric vaccines without significantly impairing the immune response to any vaccine while maximizing coverage and minimizing cost.

PATH-sponsored studies have confirmed that CD-JEV and measles vaccine could be administered concomitantly. However, no data currently exist on the simultaneous administration of CD-JEV and MMR. The proposed non-inferiority study aims to provide evidence that co-administration of MMR and CD-JEV does not adversely affect safety or immunogenicity. With the use of MMR increasing, this study will provide critical data for policy makers considering co-administration of MMR and CD-JEV.

## 2.1. Study Objectives

### 2.1.1. Primary Objective

1. To demonstrate the non-inferiority of MMR vaccination in terms of measles immunogenicity when administered concomitantly to infants 9 months of age with CD-JEV compared to administration alone, as assessed by the rate of seropositivity 56 days after vaccination.

   Measles immunogenicity will be assessed by the proportion (or percentage) of participants with demonstrated seropositivity for measles at 56 days post-vaccination. Seropositivity will be defined by a concentration of ≥120 mIU/mL of anti-measles neutralizing antibody titer, as measured by the plaque reduction neutralization test (PRNT) (dilution converted to concentration using the 3rd International Standard Reference serum).

2. To demonstrate the non-inferiority of MMR vaccination in terms of rubella immunogenicity when administered to infants 9 months of age concomitantly with CD-JEV compared to administration alone, as assessed by the rate of seropositivity 56 days after vaccination.

Rubella immunogenicity will be assessed by the proportion (or percentage) of participants with demonstrated seropositivity for rubella at 56 days post-vaccination. Seropositivity will be defined as anti-rubella Immunoglobulin G (IgG) concentration of ≥10 IU/mL (corresponding to an optical density ratio ≥1.10) using a commercial IgG enzyme-linked immunosorbent assay (ELISA) manufactured by Wampole Laboratories, Princeton, NJ.

### 2.1.2. Secondary Objectives

1. To demonstrate the non-inferiority of MMR vaccination in terms of mumps immunogenicity when administered to infants 9 months of age concomitantly with CD-JEV compared to administration alone, as assessed by the rate of seropositivity 56 days after vaccination.

   Mumps vaccine immunogenicity will be evaluated by the presence of serum anti-mumps IgG antibody. Seropositivity will be defined as an OD ratio ≥1.10 using a commercial ELISA manufactured by Zeus Scientific, Inc., Branchburg, NJ.

2. To evaluate geometric mean concentration (GMC) for serum anti-measles neutralizing antibody concentration and anti-rubella IgG GMC at 56 days post-vaccination, comparing when MMR vaccine is co-administered with CD-JEV to when MMR vaccine is given alone.

3. To evaluate anti-measles, anti-mumps, and anti-rubella seroconversion rates 56 days after vaccination, comparing when MMR vaccine is co-administered with CD-JEV to when MMR vaccine is given alone. For participants seronegative at baseline, seroconversion will be defined as a change to seropositive status (as defined above). For participants seropositive for measles or rubella at baseline, seroconversion will be defined as a four-fold rise in concentration.

4. To compare the anti-JE seropositivity rate 28 days after CD-JEV vaccination administered concurrently with MMR vaccine to CD-JEV administered alone. Anti-JE seropositivity will be defined as an anti-JE serum neutralizing antibody titer of ≥1:10, as measured by JE PRNT-50.

5. To describe geometric mean titer (GMT) for anti-JE neutralizing antibody titer 28 days after CD-JEV vaccination when MMR vaccine is co-administered compared to when CD-JEV is administered alone.

6. To describe the safety profiles of CD-JEV and MMR vaccine when given concurrently and separately, as assessed by immediate reactions occurring within 30 minutes, solicited injection site and systemic adverse reactions occurring

within 14 days, unsolicited adverse events (AEs) occurring within 28 days, and SAEs throughout the course of the study.

#### 2.1.3. Exploratory Objectives

To assess the effect of pre-existing anti-dengue antibody on CD-JEV immunogenicity. Pre-existing anti-dengue antibody will be measured first by the presence of anti-dengue IgG in ELISA and secondly by anti-dengue serum neutralizing antibody titer of ≥1:10 as measured by dengue PRNT-50.

### 2.2. Study Design

This study will be a phase 4 open-label trial in which 628 Philippine infants 9 months of age will be randomized 1:1 to one of two arms:

- Group 1: 314 children receiving one dose of CD-JEV vaccine and one dose of MMR vaccine concurrently at enrollment; Group 1 will also receive a second dose of MMR per the routine immunization schedule at D84 (12 months of age).
- Group 2: 314 children receiving one dose of MMR vaccine at enrollment and one dose of CD-JEV 56 days later. Group 2 will receive a second dose of MMR per the routine immunization schedule at D84 (12 months of age).

Following receipt of study vaccine, infants will be monitored for immediate reactions during the first 30 minutes, solicited injection site and systemic adverse reactions for 14 days, unsolicited adverse events for 28 days, and serious adverse events throughout the duration of the study. All safety events will be identified or observed by study staff and/or reported by a parent. Solicited adverse reactions will be recorded by the parent on diary cards or assessed during clinic visits. Study staff will make a follow-up call to the participant's parent/guardian 2 days post vaccination (+3 days) to check on the welfare of the participant and ensure the diary card is being completed. The parent will return for a clinic visit 14 days post vaccination (+3 days) for the study staff to review the diary card, conduct safety evaluations, and review any adverse events. Information regarding unsolicited AEs will be collected during any scheduled or unscheduled clinic visit. Events will be graded for severity and assessed for relatedness to vaccination by the study clinician.

The study will be open-label; no placebo control will be employed. Due to the unequal number of injections per visit in each study arm, safety assessments will be unblinded.

Blood samples for serologic testing will be collected on Day 0, Day 28 (+7), and Day 56 (+7) for Group 1; and Day 0, Day 56 (+7), Day 84 (+7) for Group 2.

Baseline serology will be tested for antibodies to JE, measles, mumps, rubella, and dengue virus. Antibody responses to CD-JEV will be tested 28 (+7) days after vaccination, while antibody responses to the first dose of MMR will be tested 56 (+7) days after vaccination. Specifically, antibodies to measles, mumps, and rubella will be tested on Day 0 and Day 56 (+7) for both Group 1 and Group 2; antibodies to JE will be assessed on Day 0 and Day 28 (+7) for Group 1 and on Day 56 (+7) and Day 84 (+7) for Group 2; antibodies to dengue will be tested on Day 0 for Group 1 and on Day 56 (+7) for Group 2. All laboratory testing will be conducted in a blinded fashion.

Except for a screening visit which may occur on Day 0 or before, a total of 9 visits is planned for participants in Group 1 and a total of 11 visits is planned for participants in Group 2. The duration of study participation for each participant is 4 months. The duration of the study is expected to be 7 to 8 months, dependent upon the duration of enrollment period. The schedule of study visits and activities is presented in Table 1 below.

The study will be conducted at Putatan Health Center and Bayanan Health Center located within the study area of the Research Institute for Tropical Medicine, Manila, Philippines.

Table 1. Outline of study visits and activities

| | Group 1 | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | On or before D0 | D0 | D2 (+3) | D14 (+3) | D28 (+7) | D56 (+7) | D84 (+7) | D86 (+3) | D98 (+3) | D112 (+7) |
| Obtain informed consent | X | | | | | | | | | |
| Collect baseline demographic information | | X | | | | | | | | |
| Collect/review medical history | | X | | | | | | | | |
| Perform physical examination | | X | | X | X | X | X | | X | X |
| Check/confirm inclusion/exclusion criteria | | X | | | | | | | | |
| Collect serum for serology testing | | X | | | X | X | | | | |
| Administer dose of MMR | | X | | | | | X | | | |
| Administer dose of CD-JEV | | X | | | | | | | | |
| Observe for immediate reactions for 30 minutes | | X | | | | | X | | | |
| Provide diary card to parent/guardian | | X | | | | | X | | | |
| Call participant to inquire about child's well-being | | | X | | | | | X | | |
| Collect diary card and review reported adverse events | | | | X | | | | | X | |
| Record reported adverse events | | X | | | X | X | X | | | X |
| Record reported serious adverse events | | X | X | X | X | X | X | X | X | X |
| Exit participant from study | | | | | | | | | | X |

| | Group 2 | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | On or before D0 | D0 | D2 (+3) | D14 (+3) | D28 (+7) | D56 (+7) | D58 (+3) | D70 (+3) | D84 (+7) | D86 (+3) | D98 (+3) | D112 (+7) |
| Obtain informed consent | X | | | | | | | | | | | |
| Collect baseline demographic information | | X | | | | | | | | | | |
| Collect/review medical history | | X | | | | | | | | | | |
| Perform physical examination | | X | | X | X | X | | X | X | | X | X |
| Check/confirm inclusion/exclusion criteria | | X | | | | | | | | | | |
| Collect serum for serology testing | | X | | | | X | | | X | | | |
| Administer dose of MMR | | X | | | | | | | X | | | |
| Administer dose of CD-JEV | | | | | | X | | | | | | |
| Observe for immediate reactions for 30 minutes | | X | | | | X | | | X | | | |
| Provide diary card to parent/guardian | | X | | | | X | | | X | | | |
| Call participant to inquire about child's well-being | | | X | | | | X | | | X | | |
| Collect diary card and review reported adverse events | | | | X | | | | X | | | X | |
| Record reported adverse events | | X | | | X | X | | | X | | | X |
| Record reported serious adverse events | | X | X | X | X | X | X | X | X | X | X | X |
| Exit participant from study | | | | | | | | | | | | X |

### 2.3. Sample Size and Power

An enrollment size of 628 participants (314 per treatment group) was targeted to achieve an overall power of 90% (approximate individual power of 95% for each co-primary objective). This estimation is based upon a non-inferiority margin of 10% and a one-sided type-one error rate of 2.5% or less, and assumes (based on historical data) a 95% seropositivity rate for measles vaccine, a 90% seropositivity rate for rubella vaccine, and approximately 20% non-evaluable rate (exclusion for baseline seropositivity and loss to follow-up) at 56 days post-vaccination. The sample size calculations were based on the Farrington-Manning score test.

## 3. General Considerations for Data Analyses

All statistical tests except for non-inferiority will be two-sided. P-values will be recorded to 4 decimal places. All analyses will be conducted using SAS version 9.4.

## 3.1. Analysis Set

All immunogenicity analyses and summaries will be performed on a per-protocol basis which will be considered as the primary approach to immunogenicity analyses. Supportive intention-to-treat immunogenicity analyses will also be conducted on all enrolled participants who received at least one dose of study vaccines and have at least one post-vaccination serology result. Safety analyses will be conducted on an intention-to-treat basis. In the event that there are participants who do not receive the vaccine(s) to which they were randomized, safety analyses will be performed on the safety analysis set as well. Analyses set definitions are provided below.

### 3.1.1. Intention-to-Treat Analysis Set

The Intention-To-Treat (ITT) set will include all participants who received a study vaccine. Participants in the ITT data set will be analyzed in the group to which participants have been assigned (not based on the actual vaccine received). Participants who withdraw or are terminated from the study will be included in the ITT data set until the time of withdrawal or termination. Participants in the ITT analysis set who have at least one post-vaccination serology result will be included in the supportive immunogenicity analysis.

### 3.1.2. Safety Analysis Set

The Safety Analysis Set (SAS) will include all participants who received a study vaccine and have at least one post-vaccination safety result. Participants in the safety data set will be analyzed based on the actual vaccine received rather than the group to which they have been randomized.

### 3.1.3. Per-Protocol Analysis Set

The Per-Protocol (PP) sets will include the participants in the ITT set who are compliant with the protocol. This data set will be used for immunogenicity analysis only. The antigen specific PP sets are defined as follows.

***Measles Per-Protocol Analysis Set (measles-PP)***

The measles-PP analysis set will be based on participants who meet all of the following criteria:

- Fulfilled the eligibility (inclusion/exclusion) criteria.
- Received all study vaccines as assigned.
- Provided a valid measles serology laboratory result at baseline and at 56 days post-vaccination with MMR vaccine, with specimens taken within the windows indicated in Table 1.
- Did not take any prohibited concomitant medication within 28 days post-vaccination.
- Did not show seropositivity for measles at baseline.

***Mumps Per-Protocol Analysis Set (mumps-PP)***

The mumps-PP analysis set will be based on participants who meet all of the following criteria:

- Fulfilled the eligibility (inclusion/exclusion) criteria.
- Received all study vaccines as assigned.
- Provided a valid mumps serology laboratory result at baseline and at 56 days post-vaccination with MMR vaccine, with specimens taken within the windows indicated in Table 1.
- Did not take any prohibited concomitant medication within 28 days post-vaccination.
- Did not show seropositivity for mumps at baseline.

***Rubella Per-Protocol Analysis Set (rubella-PP)***

The rubella-PP analysis set will be based on participants who meet all of the following criteria:

- Fulfilled the eligibility (inclusion/exclusion) criteria.
- Received all study vaccines as assigned.
- Provided a valid rubella serology laboratory result at baseline and at 56 days post-vaccination with MMR vaccine, with specimens taken within the windows indicated in Table 1.
- Did not take any prohibited concomitant medication within 28 days post-vaccination.
- Did not show seropositivity for rubella at baseline.

***JE Per-Protocol Analysis Set (JE-PP)***

The JE-PP analysis set will be based on participants who meet all of the following criteria:

- Fulfilled the eligibility (inclusion/exclusion) criteria.
- Received all study vaccines as assigned.
- Provided a valid JE serology laboratory result at baseline and at 28 days post-vaccination with CD-JEV vaccine, with specimens taken within the windows indicated in Table 1.
- Did not take any prohibited concomitant medication within 28 days post-vaccination.
- Did not show seropositivity for JE at baseline.

## 3.2. Adjustment for Covariates

No adjustments for covariates are planned for the primary analysis. For the comparison of ant-measles and anti-rubella GMCs and anti-JE GMT between treatment groups, baseline antibody concentration/titer will be included in the analysis as covariate. In addition, age prior to vaccination with CD-JEV will be evaluated for inclusion as covariate for the comparison of anti-JE GMT between study groups.

## 3.3. Missing Data and Outliers

### 3.3.1. Missing Data

All descriptive statistics will be performed on subjects with available data. Non-missing serology data are considered validated. Missing serology data are considered non-retrievable. Missing serology data will not be imputed and will be analyzed as if they were randomly missing.

If severity or relationship to a study vaccine for a reported adverse event (AE) or serious adverse event (SAE) is missing, an independent category "Missing" will be reported.

If start date associated with a reported concomitant medication, AE or SAE is incomplete or missing, the following rules will be applied:

- If the day of the date is missing, use the day of the date of vaccination if start month and year are the same as those of date of vaccination; otherwise use the 15$^{th}$ day of the month.
- If either month or year is missing, no imputations will be done.

If stop date for a concomitant medication, AE or SAE is incomplete or missing, the medication will be considered ongoing.

If date of withdrawal/study completion is completely missing, it will not be imputed.

If date of study vaccination is incomplete or missing, the following rules will be applied:

- If day is missing, use the day of the corresponding visit at which the vaccination is scheduled if start month and year are the same as those of date of the corresponding visit; otherwise use the 15$^{th}$ day of the month.
- If either month or year is missing, no imputation will be done.

### 3.3.2. Outliers

No data will be excluded from the primary and secondary analyses, including any outliers. Transformation on antibody responses during analysis can reduce the impact of the outliers. For continuous immunogenicity measures, non-parametric comparisons may be used as a sensitivity analysis if necessary.

## 3.4. Data Handling Conventions and Transformations

For Group 1, baseline antibodies to measles, rubella, mumps, JE, and dengue will be tested on Day 0. For Group 2, baseline antibodies to measles, rubella, and mumps will be assessed on Day 0 and baseline antibodies to JE and dengue will be tested on Day 56.

The antibody concentrations/titers to measles, rubella, JE, and dengue will be transformed using $\log_{10}$ transformation. Data will be back-transformed to the original scale for presentation.

Numerical numbers will be presented to one decimal place if applicable.

### 3.5. Multicenter Studies

The primary and secondary analyses will not be adjusted by site.

### 3.6. Multiple Comparisons/Multiplicity

No multiplicity adjustments will be performed as we expect to show if both co-primary objectives will be achieved at the same time.

### 3.7. Timing of Analyses

No formal interim analysis will be performed for this study. Due to an expected delay in MMR testing, JE testing will be completed first. According to this order of available serology testing results, two independent final analyses will be conducted stepwise as follows:

- The first final analysis will include all safety data and JE immunogenicity results. The estimates of the JE immunogenicity endpoints and safety endpoints and statistical tests associated with relevant secondary objectives and exploratory objective will be provided. This analysis will be performed after anti-JE antibody results are available and database is cleaned and locked at the end of the study.
- The second final analysis will included all MMR immunogenicity results. The estimates of the MMR-related immunogenicity endpoints and statistical tests associated with primary and relevant secondary objectives will be presented. This analysis will be performed as soon as MMR results are available, cleaned, and locked.

## 4. Subject Disposition

### 4.1. Subject Enrollment

The number of subjects screened and the number of subjects excluded will be tabulated. The number of subjects randomized will be tabulated by study group.

### 4.2. Disposition of Subjects

Subject disposition will be summarized by study group. The following information will be tabulated.

- The number and proportion of subjects in ITT, PP, and safety analysis sets.
- The number and proportion of subjects who were randomized but received no vaccines.
- The number and proportion of subjects who are excluded from the ITT analysis and reasons for exclusion.
- The number and proportion of subjects who are excluded from the PP analysis and reasons for exclusion.
- The number and proportion of subjects who completed the study visit.
- The number and proportion of subjects who have blood collection done at each applicable visit.
- The number and proportion of subjects who have valid serology results at each applicable visit.

Listings of subject disposition and status, exclusions from the ITT analysis, exclusions from the PP analysis, and exclusions from the safety analysis will be provided.

### 4.3. Withdrawals of Subjects

Participants may withdraw from the study at any time and for any reason. The investigator may withdraw participants from the study for various reasons. The number of withdrawals and the reasons for the withdrawals will be summarized by study group.

## 5. Subject Characteristics

### 5.1. Baseline Characteristics

Demographic and baseline characteristics including age, gender at birth, weight, and height will be summarized by study group for the ITT and PP populations. Age will be calculated in months rounding to the precision of one decimal place using the following formula:

Age (months) = [(Date of Day 0 – Date of Birth) x 12]/365.25

Age, weight, and length will be described by number of subjects with data, mean, standard deviation (SD), median, minimum, and maximum. Gender at birth will be described by number and proportion of subjects in each study group. Denominators for proportions will be the number of subjects with non-missing values. In order to confirm whether study groups of our interest are similar in terms of demographic and baseline characteristics, T-test will be used to compare age, weight, and length and Fisher's Exact Test will be used to compare gender at birth.

In addition, vital signs, immunization history, medical history, pre-existing conditions, and physical exam collected on Day 0 will be summarized by study group.

Baseline serology results in terms of antigen-specific seropositivity will be provided as part of the immunogenicity results. In addition, GMCs/GMT for the antibody concentrations/titers to measles, rubella, JE, and dengue will be provided at baseline as well.

### 5.2. Subject Characteristics at Each Visit

Vital signs (height, weight, axillary temperature, respiratory rate, blood pressure, pulse rate, weight for height z-score < -3 (yes or no) and physical exam (general appearance, head, eyes, ears, nose, throat, lymph nodes, cardiovascular/heart, respiratory/lungs, abdomen, musculoskeletal/extremities, skin, neurological/central, endocrine, health status) collected at other visits will be summarized for ITT population by study group.

Listings of demographics, immunization history, medical history, pre-existing condition, vital signs, and physical exam at each applicable time point will be provided at participant level.

### 5.3. Concomitant Medications

Concomitant medications collected during study will be summarized in terms of anatomical therapeutic chemical (ATC) classification and preferred drug name for the ITT population by study group. The preferred drug name, ATC classification, route, dose, units, frequency, start and stop dates, and duration of the medications will be listed at participant-level.

The duration of a concomitant medication will be computed as follows:

Duration (days) = Stop Date – Start Date +1

If the stop date is completely missing, the particular medication will be considered to be ongoing and the duration will not be calculated.

## 6. Immunogenicity Analyses

Immunogenicity analyses will primarily be performed on PP analysis set. Supportive immunogenicity analyses will also be conducted on ITT analysis set.

### 6.1. Primary Immunogenicity Endpoints

- Proportion (or percentage) of participants with demonstrated seropositivity for measles at 56 days post-vaccination. Seropositivity will be defined as a post-

vaccination concentration of ≥ 120 mIU/mL, as measured by PRNT (using the 3rd International Standard Reference serum to convert dilutions to concentrations).

- Proportion (or percentage) of participants with demonstrated seropositivity for rubella at 56 days post-vaccination. Seropositivity will be defined as a post-vaccination concentration of ≥ 10 IU/mL (corresponding to an OD ratio ≥ 1.10 using a commercial ELISA manufactured by Wampole Laboratories, Princeton, NJ).

## 6.2. Analysis of Primary Immunogenicity Endpoints

**Primary Hypotheses**

To demonstrate the non-inferiority of MMR vaccination in terms of measles and rubella immunogenicity when administered concomitantly with CD-JEV to infants 9 months of age compared to administration alone, the following co-primary hypotheses will be tested:

$H_{0M}$: ($P_{(M1)}$ minus $P_{(M2)}$) ≤ –10%
$H_{1M}$: ($P_{(M1)}$ minus $P_{(M2)}$) > –10%

and

$H_{0R}$: ($P_{(R1)}$ minus $P_{(R2)}$) ≤ –10%
$H_{1R}$: ($P_{(R1)}$ minus $P_{(R2)}$) > –10%

where $P_{(M1)}$ refers to the percentage of participants with seropositivity for measles in Group 1 and $P_{(M2)}$ refers to the percentage of participants with seropositivity for measles in Group 2 and where $P_{(R1)}$ refers to the percentage of participants with seropositivity for rubella in Group 1 and $P_{(R2)}$ refers to the percentage of participants with seropositivity for rubella in Group 2.

**Analysis Methods**

The proportion of participants with seropositivity for measles and rubella at 56 days post-vaccination will be presented with exact 95% CI using Clopper-Person method by study group.[1]

The comparison between Group 1 and Group 2 will be tested for non-inferiority. Non-inferiority will be achieved when both $H_{0M}$ and $H_{0R}$ are rejected, that is, if the lower limit of the two-sided 95% CI for the difference in percentages of participants with seropositivity (concurrent administration minus separate administration) at 56 days post-vaccination is > -10% for both measles and rubella results. The 95% CI will be calculated using Farrington-Manning score method.[2]

## 6.3. Secondary Immunogenicity Endpoints

- Proportion (or percentage) of participants with demonstrated seropositivity for mumps at 56 days post-vaccination. Seropositivity will be defined as an optical

density ratio ≥ 1.10 using a commercial ELISA manufactured by Zeus Scientific, Inc., Branchburg, NJ.

- Geometric mean concentration (GMC) for anti-measles neutralizing antibody concentration at 56 days post-vaccination
- GMC for anti-rubella IgG antibody concentration at 56 days post-vaccination
- Seroconversion rates for measles at 56 days post-vaccination, defined as the proportion of participants with a change in serostatus from negative to positive 56 days after vaccination or a four-fold rise in concentration 56 days after vaccination if seropositive for measles at baseline
- Seroconversion rates for mumps at 56 days post-vaccination, defined as the proportion of participants with a change in serostatus from negative to positive 56 days after vaccination
- Seroconversion rates for rubella at 56 days post-vaccination, defined as the proportion of participants with a change in serostatus from negative to positive 56 days after vaccination or a four-fold rise in concentration 56 days after vaccination if seropositive for rubella at baseline.
- Proportion (or percentage) of participants with demonstrated seropositivity for JE at 28 days post-vaccination with CD-JEV. Seropositivity will be defined as a JE serum neutralizing antibody titer of ≥ 1:10, as measured by JE PRNT-50.
- Geometric mean titer (GMT) for serum neutralizing antibody titer to JE virus at 28 days post-vaccination

### 6.4. Analysis of Secondary Immunogenicity Endpoints

- Proportions (or percentage) of participants with seropositivity for mumps at 56 days post-vaccination between Group 1 and Group 2 will be compared using a non-inferiority test. Non-inferiority of Group 1 to Group 2 in terms of seropositivity for mumps will be demonstrated if the lower limit of the two-sided 95% CI for the difference of seropositivity rates between the two groups (concurrent administration minus separate administration) at 56 days post-vaccination is > -10%. The 95% CI will be calculated using the Farrington-Manning method. The proportion of participants with seropositivity for mumps at 56 days post-vaccination will be presented with exact 95% CI by study group.
- The ratio of anti-measles GMCs between the two groups (ratio of concurrent administration to separate administration) along with 95% CI of the ratio at 56 das post-vaccination will be obtained using analysis of covariance (ANCOVA) approach that includes $\log_{10}$-transformed antibody concentration as the dependent variable and the treatment group as the explanatory variable adjusted for $\log_{10}$-transformed baseline antibody concentration. The anti-measles GMC with its 95% CI will be provided by study group. The same approach will be applied to anti-rubella GMCs.
- The 95% CI of the difference in anti-measles seroconversion rates between the two groups (concurrent administration minus separate administration) at 56 days post-vaccination will be calculated using the Farrington-Manning method. The

seroconversion rate will be presented with exact 95% CI by study group. The sample approach will be employed to anti-mumps and anti-rubella seroconversion rates.

- The 95% CI of the difference in proportion (or percentage) of participants with seropositivity for JE between the two groups (concurrent administration minus separate administration) at 28 days post-vaccination with CD-JEV will be calculated using the Farrington-Manning method. The JE seropositivity rate will be calculated with exact 95% CI by study group.
- The ratio of anti-JE GMTs between the two groups (ratio of concurrent administration to separate administration) along with 95% CI of the ratio at 28 days post-vaccination with CD-JEV will be obtained using analysis of covariance (ANCOVA) approach that includes $\log_{10}$-transformed antibody titer as the dependent variable and the treatment group as the explanatory variable adjusted for $\log_{10}$-transformed baseline antibody titer. The age prior to vaccination with CD-JEV will be evaluated for inclusion as covariate in ANCOVA model. The anti-JE GMT with its 95% CI will be summarized by study group.

### 6.5. Exploratory Analyses

#### 6.5.1. Immunogenicity Endpoints of Exploratory Analysis

- Proportion (or percentage) of participants with presence of anti-dengue IgG as measured by ELISA prior to vaccination with CD-JEV.
- Proportion (or percentage) of participants with anti-dengue serotype 1-, 2-, 3-, and 4-specific serum neutralizing antibody titer of $\geq$ 1:10 as measured by dengue PRNT-50 prior to vaccination with CD-JEV.

#### 6.5.2. Exploratory Immunogenicity Analysis

The proportions along with 95% exact CI for anti-dengue antibody response will be calculated by study group.

To assess the effect of pre-existing anti-dengue antibody prior to vaccination with CD-JEV on CD-JEV immunogenicity 28 days following immunization, a two-way cross tabulation of anti-dengue antibody status (non-presence/presence, negative/positive) prior to vaccination with CD-JEV and anti-JE antibody response (negative/positive) 28 days post-vaccination will be provided. The association between pre-existing anti-dengue antibody and anti-JE antibody response post-vaccination will be tested by Fisher's Exact Test.

## 7. Safety Analyses

### 7.1. Safety endpoints

- Frequency count and proportion of participants reporting immediate reaction occurring within 30 minutes of each vaccination.
- Frequency count and proportion of participants reporting solicited signs and symptoms occurring within 14 days of each vaccination.
- Frequency count and proportion of participants reporting unsolicited AEs occurring within 28 days of each vaccination.
- Frequency count and proportion of participants reporting SAEs occurring throughout the course of the study.

### 7.2. Analysis of Safety endpoints

- The number and proportion of participants with at least one occurrence of an immediate reaction occurring within 30 minutes of each vaccination along with exact 95% CI of the proportion will be tabulated by study group. The severity, relationship to the vaccination, status at 30 minutes (ongoing or not), medication given (yes or no), reported as SAE (yes or no), and total number of the immediate reactions will be summarized by study group as well. Proportion of participants will be based on the number of participants who are in ITT analysis set or the safety analysis set when applicable. In addition, the immediate reaction will be listed at participant-level.
- The number and proportion of participants with at least one occurrence of local reactions (ecchymosis, erythema, edema, induration, and pain/tenderness) and with at least one occurrence of systemic reactions (fever, rash, cough, runny nose, change in eating habits, diarrhea, sleepiness, irritability, unusual crying, and vomiting) occurring greater than 30 minutes after receipt of each vaccination through 14 days post vaccination will be summarized by study group along with exact 95% CI of the proportion. Each specific local or systemic reaction will be summarized by severity for each study group. A listing including the local and systemic reactions at the participant-level will be provided as well.
- The number and proportion of participants with at least one unsolicited AE occurring within 28 days of each vaccination along with exact 95% CI of the proportion will be presented by study group. The AEs will be summarized by maximum severity and relationship to each vaccination for each study group. The total number, maximum severity, relationship to the vaccination, onset date, status/outcome, duration for resolved cases (resolution date – onset date + 1), and medication given (yes or no) of AEs will be also summarized by study group. Summaries will be repeated for AEs found to be related to the vaccination. A listing of AEs at participant-level will be presented.
- The number and proportion of participants with at least one SAE occurring throughout the course of the study along with exact 95% CI of the proportion will be presented by study group. The SAEs will be summarized by maximum severity and relationship to each vaccination for each study group. The total number, maximum severity, relationship to the vaccination, onset date, status/outcome, duration for resolved cases (resolution date – onset date + 1), and medication given

(yes or no) of SAEs will be also summarized by study group. Summaries will be repeated for SAEs found to be related to the vaccination. A listing of SAEs at participant-level will be presented.

## 8. Reference

1. Clopper, C. J., and Pearson, E. S. (1934), "The Use of Confidence or Fiducial Limits Illustrated in the Case of the Binomial," *Biometrika* 26, 404–413.

2. Farrington CP, Manning G. Test statistics and sample size formulae for comparative binomial trials with null hypothesis of non-zero risk difference or non-unity relative risk. Stat Med 9(12):1447–1454. 1990.