Statistical Analysis Plan

Protocol Title	Assessment of long term immunogenicity of Japanese encephalitis live attenuated SA-14-14-2 vaccine in previously vaccinated Bangladesh children and antibody response and safety to a booster dose
Protocol Number	JEV07 PR-15036
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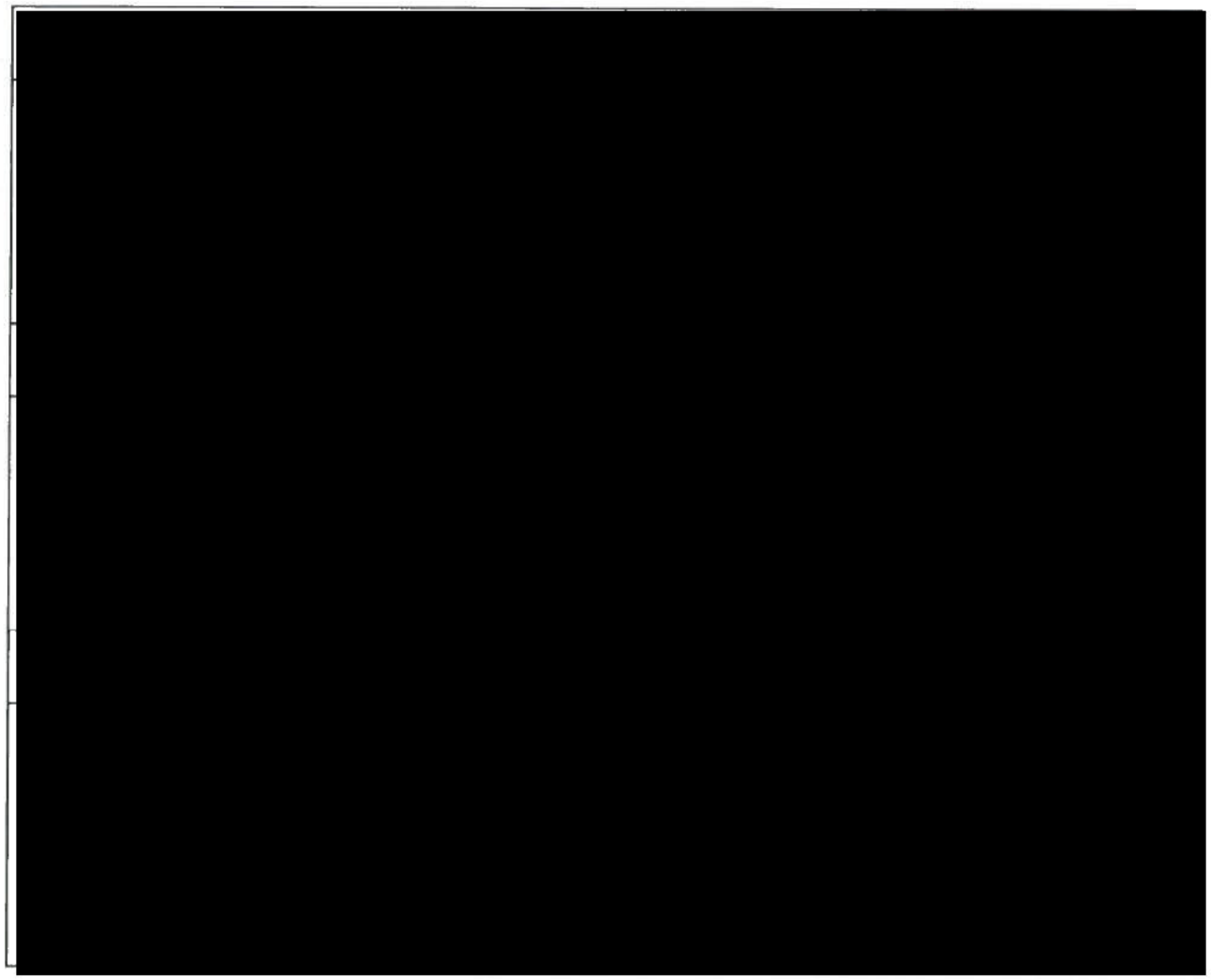
Statistical Analysis Plan Signature Page



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1. List of Abbreviations

AE	Adverse Event
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
FSC	Fixed site clinic
GMP	Good manufacturing practice
GMT	Geometric Mean Titer
HDSS	Health and demographic surveillance system
icddr, b	International Center for Diarrheal Diseases Research, Bangladesh
IgM	Immunoglobulin M
ITT	Intent to treat
JE	Japanese encephalitis
PP	Per protocol
SAE	Serious Adverse Event
SD	Standard Deviation

2. Introduction

In 2012 PATH sponsored a double-blind, randomized controlled trial (PATH JEV05; icddr,b Protocol number 11050) among 818 Bangladeshi infants aged between 10 and 12 months to compare the immunogenicity of three lots of live attenuated SA 14-14-2 Japanese encephalitis vaccine (CD-JEV) manufactured in the Chengdu Institute of Biological Products (CDIBP) newly-constructed Good Manufacturing Practice (GMP) facility and one lot of CD-JEV manufactured in the pre-existing CDIBP facility.

The proposed study will enroll Bangladeshi children who previously participated in JEV05 to measure the persistence of the antibody response and determine if and when a booster dose of CD-JEV may be needed to maintain protective antibody levels. In addition, this study will determine the safety of a second dose of CD-JEV in this population.

2.1. Study Objectives

2.1.1. Primary Objective

To assess the long-term antibody response three and four years after primary CD-JEV vaccination of children who received a single dose of vaccine between 10 and 12 months of age.

2.1.2. Secondary Objectives

- To evaluate the antibody response following a booster dose of CD-JEV given at five years of age, among children who previously received a single dose of CD-JEV between 10 and 12 months of age.
- To determine the safety profile of a CD-JEV booster dose given to children four years after initial vaccination.

2.1.3. Exploratory Objectives

To describe the antibody response to a booster dose by differentiating between a primary and secondary antibody response at 7 days post-booster dose.

2.2. Study Design

This is a phase 4 open-label trial enrolling Bangladeshi children who previously participated in JEV05. Eligible participants who still live in the area will be identified using the local health and demographic surveillance system (HDSS) and asked to participate. Children will be divided into two groups based on whether they received vaccine from the old or new facility in the JEV05 study:

- Group A (up to 655 eligible): children randomized to Groups 2-4 (new facility) in JEV05.
- Group B (up to 163 eligible): children randomized to Group 1 (old facility) in JEV05.

A 2 mL blood sample will be collected from all JEV05 participants enrolled into this study three (Visit 01) and four (Visit 02) years after initial vaccination, respectively. The sera will be tested using a 50% plaque reduction neutralization test (PRNT-50); anti-Japanese encephalitis (JE) neutralizing antibody titer ≥ 1:10 will be considered seroprotective.

Four years after receiving the initial dose of CD-JEV, eligible participants will be vaccinated with a single subcutaneous booster dose of CD-JEV. To evaluate the persistence of immunological memory, the response to this booster dose will be measured in a serum sample collected 7 (Visit 09) and 28 days (Visit 10) after vaccination. This sample will be tested for anti-JE neutralizing antibodies.

Following the booster dose, children will be monitored for immediate reactions during the first 30 minutes, solicited injection site and systemic adverse reactions for 7 days (Visit 02 – Visit 08), unsolicited adverse events (AEs) and serious adverse events (SAEs) for 28 days. All safety events will be identified or observed by study staff during home visits, clinic visits, and/or reported by a parent or legal guardian. Solicited local and systemic adverse reactions will be assessed through home visits 1 through 6 days following vaccination, and a fixed site clinic (FSC) visit 7 days following vaccination. Information regarding unsolicited AEs occurring in the 28 days following vaccination will be collected and recorded on a standard questionnaire during clinic visits. Events will be graded for severity and assessed for relatedness to vaccination by the investigator.

A total of 10 visits is planned for this study. The schedule of study visits and activities is presented in Table 1 below.

No placebo control will be employed in this study and safety assessments will be unblinded.

The study will be conducted in the same sites as JEV05, i.e., Matlab and Mirpur (Dhaka).

Table 1. Outline of study visits and activities

	Y3 after JEV05 ±3 months after enrollment in JEV05	Y4 after JEV05			
	Visit 01 (D0)	Visit 02 (D365±28)	Visit 03 through Visit 08 (D366-371±0)	Visit 09 (D372+3)	Visit 10 (D393±7)
Obtain informed consent	X				
Collect baseline demographic information	X				
Collect/review medical history	X	X			
Perform physical exam	X	X		X	X
Check inclusion/exclusion criteria	X	X			
Collect serum for long term response	X	X			
Administer booster dose of CD-JEV		X			
Collect serum for booster response				X	X
Observe for immediate reactions for 30 minutes		X			
Inquire about child's well being			X	X	X
Record reported adverse events		X	X	X	X
Record serious adverse events		X	X	X	X
Exit participant from study					X

2.3. Sample Size and Power

Since the study aims at following up with the participants of the JEV05 study who received a single dose of CD-JEV between 10 and 12 months of age, a maximum of 818 former JEV05 participants (up to 655 in Group A and up to 163 in Group B) is expected to be enrolled in this study. We anticipate an attrition rate from 10% to 40% in both groups, i.e. 10% to 40% of the subjects in Group A and Group B may not be enrolled into this study.

The precision (width) of the 95% confidence interval (CI) of the seroprotection rate before a booster vaccination is calculated based upon different assumptions on attrition rate and expected seroprotection rates and provided in Table 2.

Table 2. Precision of the 95% CI of the seroprotection rate according to attrition rate and seroprotection rate in each study group

Study Group	Expected seroprotection rate	Attrition rate			
		10%	20%	30%	40%
Group A	40%	0.079	0.084	0.089	0.096
	50%	0.080	0.085	0.091	0.098
	60%	0.079	0.084	0.089	0.096
	70%	0.074	0.078	0.084	0.090
	80%	0.065	0.068	0.073	0.079
Group B	40%	0.157	0.166	0.177	0.191
	50%	0.160	0.169	0.181	0.195
	60%	0.157	0.166	0.177	0.191
	70%	0.147	0.156	0.166	0.180
	80%	0.129	0.137	0.146	0.158

3. General Considerations for Data Analyses

All statistical tests will be two-sided with a significance level of 0.05. P-values will be recorded to 4 decimal places. All analyses will be conducted using SAS.

3.1. Analysis Set

All immunogenicity analyses will be performed on intention-to-treat (ITT) analysis set, which will be considered as the primary approach for immunogenicity analyses. The immunogenicity analyses will be also conducted on per protocol (PP) analysis set. Safety analysis will be conducted on participants who received a booster dose among the ITT population. Each analysis set is specified below.

3.1.1. Intention-to-Treat Analysis Set

For immunogenicity analysis on long-term assessment of the anti-JE antibody response to CD-JEV, the ITT analysis set will include all enrolled participants in this study whether the participants receive a booster dose of CD-JEV or not. The participants who have at least one serology result in Year 3 (Visit 01) or Year 4 (Visit 02) prior to the booster dose will be included in the long-term assessment.

For the immunogenicity analysis on antibody response following a booster dose of CD-JEV, the ITT analysis set will include those participants who receive the booster dose and have at least one serology result on 7 days (Visit 09) or 28 days (Visit 10) post booster vaccination.

3.1.2. Per Protocol Analysis Set

The PP analysis set will include the participants in the ITT set who comply with the protocol. For the long-term immunogenicity assessment, participants will be included in the PP analysis set if they meet eligibility criteria at Visit 01 (Year 3), have a valid serology result in Year 3 (Visit 01) and Year 4 (Visit 02) prior to the booster vaccination, and have no significant deviation in blood collection at the two time points.

For the immunogenicity analysis on antibody response following a booster dose of CD-JEV, participants will be included in the PP set if they meet eligibility criteria for the booster vaccination, have a valid serology result in Year 4 prior to the booster vaccination, receive the booster dose, have valid serology results on 7 days (Visit 09) and 28 days (Visit 10) post the booster dose, and do not significantly deviate in the scheduled blood collection post booster vaccination.

3.1.3. Safety analysis set

The safety analysis set will include all enrolled participants who receive a booster dose and will be used to analyze all safety measurements including reactogenicity (local and systemic signs and symptoms), AEs, and SAEs collected after a booster dose of CD-JEV.

3.2. Adjustment for Covariates

No adjustment for covariates is planned. However, longitudinal analysis may be performed for the long-term assessment of the anti-JE antibody response to CD-JEV as an ad-hoc analysis if necessary. If the longitudinal analysis is conducted, duration of follow-up since the initial vaccination will be included in the analysis as covariates. Other variables (e.g., gender, study site, nutrition status) as appropriate will be evaluated for inclusion as covariates as well. The longitudinal analyses will be considered exploratory and will not replace the primary planned analysis.

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 the booster JE vaccine for a reported adverse event (AE) or serious adverse event (SAE) is missing, an independent category “Missing” will be reported.

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

If stop date for a concomitant medication/medical history (AE or SAE) is missing, the medication/medical history will be considered ongoing.

If the study termination date is missing, it will not be imputed.

If date of JE booster vaccination in incomplete or missing, the following rules will be applied:

- If day is missing, use the day of Visit 02 (Year 4) if start month and year are the same as those of date of Visit 02; otherwise use the 15th day of the month.
- If either month or year is missing, no imputation will be done.

3.3.2. Outliers

Graphic inspection for outliers will be performed. No data will be excluded from the primary and secondary analyses, including any outliers. Transformation on antibody titers 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 the secondary analyses on booster response, baseline is defined as Year 4 post primary vaccination.

The neutralizing antibody titers will be transformed using $\log_{10}$ transformation. Data will be back-transformed to the original scale for presentation.

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 most of the analyses will be descriptive in nature.

3.7. Interim Analysis

An interim analysis on immunogenicity assessment (anti-JE neutralizing antibody titer) collected at Visit 01 (Year 3 post primary immunization) will be conducted to gain knowledge on whether or not a booster JE vaccine needs to be given sooner. JE seroprotection rate, defined as the proportion of study participants with an anti-JE neutralizing antibody titer ≥ 1:10 as measured by 50% plaque reduction neutralization test (PRNT-50), and geometric mean titer (GMT) of anti-JE neutralizing antibody at Visit 01 will be summarized by study group along with 95% CI. The group comparisons in terms of seroprotection rate and GMT will be provided. The analysis will be further stratified by the seroprotection status of participants on Day 28 post primary immunization in previous JEV05 study.

4. Subject Disposition

4.1. Subject Enrollment

Expected and actual subject enrollment will be tabulated by study group. The number and proportion of invited subjects meeting and not meeting entry criteria will also be tabulated.

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 received a booster dose of CD-JEV.
- 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 are excluded from the safety analysis and reasons for exclusion.
- The number and proportion of subjects who completed the study visit, as well as subjects who discontinued the study early and reasons for discontinuation.
- 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.

5. Subject Characteristics

5.1. Baseline Characteristics

Demographic and baseline characteristics collected at Visit 01, including age, gender at birth, weight, height, body temperature, blood pressure, pulse rate, and respiratory rate will be summarized by study group for the ITT and PP populations. Age, weight, length, body temperature, blood pressure, pulse rate, and respiratory rate 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. T-test and Fisher's Exact Test will be used to confirm whether study groups of our interest are similar in terms of demographic and baseline characteristics.

In addition, immunization history, medical history, pre-existing conditions, and physical exam collected at Visit 01 will be summarized.

5.2. Subject Characteristics at Each Visit

Demographic characteristics, immunization history, medical history, pre-existing conditions, and physical exam collected at other visits will be summarized for ITT population by study group.

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

6. Immunogenicity Analyses

The primary analysis will be conducted on the available immunogenicity data.

6.1. Immunogenicity Endpoints

The immunologic endpoints to assess the long-term antibody response of primary CD-JEV vaccination are as follows:

- Seroprotection rate at Year 3 (Visit 01) and Year 4 (Visit 02) post primary immunization, defined as the proportion of study participants with an anti-JE neutralizing antibody titer ≥ 1:10 as measured by 50% plaque reduction neutralization test (PRNT-50).
- Geometric mean titer (GMT) of anti-JE neutralizing antibody at Year 3 and Year 4 post primary immunization as measured by PRNT-50.

The immunologic endpoints to evaluate the antibody response following a booster dose of CD-JEV are as follows:

- Seroprotection rate at 7 days after the booster dose (Visit 09) and 28 days after the booster dose (Visit 10), defined as the proportion of study participants with an anti-JE neutralizing antibody titer ≥ 1:10 as measured by 50% plaque reduction neutralization test (PRNT-50).
- GMT of anti-JE neutralizing antibody at 7 days and 28 days after the booster dose as measured by PRNT-50.
- Seroconversion rate at 7 days and 28 days after the booster dose. For participants seronegative (participant with an anti-JE neutralizing antibody titer < 1:10 as measured by PRNT-50) at baseline (Year 4 post primary vaccination), seroconversion is defined as a change in serostatus from negative (anti-JE neutralizing antibody titer < 1:10) to positive (anti-JE neutralizing antibody titer ≥ 1:10). For participants seropositive at baseline, seroconversion is defined as a 4-fold or higher response in anti-JE neutralizing antibody titer post booster dose in relation to pre-booster anti-JE neutralizing antibody titer.
- GMT ratio between 7 days after the booster dose and Year 4 (Visit 02) prior to booster dose and between 28 days after the booster dose and Year 4 (Visit 02) prior to booster dose.

6.2. Analysis of Immunogenicity Endpoints

To assess the long-term antibody response three and four years after primary CD-JEV vaccination, the following analyses will be conducted,

- The number and proportion of participants with an anti-JE neutralizing antibody titer ≥ 1:10 will be tabulated by study group at Year 3 and Year 4 post primary immunization. The 95% CI of the proportion will be provided using Wilson score method without continuity correction [1, 2].
- The difference in JE seroprotection rates between Groups A and B will be calculated along with the 95% CI of the difference using the Newcombe-Wilson method without continuity correction [3].
- GMTs of the anti-JE neutralizing antibody response and their associated 95% CI will be calculated at Year 3 and Year 4 post immunization.
- The GMTs will be compared between Groups A and B using t-test at each applicable time point. The ratio of the GMTs along its 95% CI will be provided.
- Reverse cumulative distribution curves will be plotted for participants as anti-JE neutralizing antibody titer (x-axis) versus the proportion of participants with a titer of at least certain level (y-axis) at Year 3 and Year 4.

To evaluate the antibody response following a booster dose of CD-JEV given at five years of age, the following analyses will be performed,

- The number and proportion of participants with an anti-JE neutralizing antibody titer ≥ 1:10 will be tabulated by study group at 7 days after the booster dose and 28 days after the booster dose. The 95% CI of the proportion will be provided using Wilson score method without continuity correction.

- The difference in JE seroprotection rates between Groups A and B will be calculated along with the 95% CI of the difference using the Newcombe-Wilson method without continuity correction.
- GMTs of the anti-JE neutralizing antibody response and their associated 95% CI will be calculated at 7 days and 28 days post the booster dose.
- The GMTs will be compared between Groups A and B using t-test at each applicable time point. The ratio of the GMTs along its 95% CI will be provided.
- The number and proportion of participants with seroconversion will be tabulated by study group at 7 days and 28 days after the booster dose. The 95% CI of the proportion will be provided using Wilson score method without continuity correction.
- The difference in seroconversion rates between Groups A and B will be calculated along with the 95% CI of the difference using the Newcombe-Wilson method without continuity correction.
- The GMTs at 7 days and 28 days post the booster dose will be further compared to the GMTs at Year 4 prior to the booster dose using a paired t-test to evaluate the magnitude of increase in GMTs by study group. The GMT ratio between 7 days post the booster dose and Year 4 and between 28 days post the booster dose and Year 4 will be provided along with it 95% CI.
- Reverse cumulative distribution curves will be plotted for participants as anti-JE neutralizing antibody titer (x-axis) versus the proportion of participants with a titer of at least certain level (y-axis) at 7 and 28 days post the booster dose.

In addition, the booster response analyses on selected immunogenicity endpoints will be repeated on subgroups defined by seroprotection status of participants at Year 4 post primary CD-JEV vaccination.

Listing of serology results corresponding to each blood collection at participant-level will be provided.

6.3. Exploratory Analyses

6.3.1. Immunogenicity Endpoints of Exploratory Analyses

The immunologic endpoints for exploratory analyses are as follows:

- GMT of anti-JE neutralizing antibody at Year 4 prior to a booster dose of CD-JEV, 7 days, and 28 days after the booster dose as measured by PRNT-50.
- GMT ratio between 7 days after the booster dose and Year 4 prior to the booster dose and between 28 days after the booster dose and Year 4.

6.3.2. Exploratory Immunogenicity Analysis

The antibody response to a booster dose of CD-JEV will be examined on the following two subgroups:

- Primed group includes participants who will be seronegative for anti-JE immunoglobulin M (IgM) prior to the booster dose (Year 4) and 7 days after the booster dose and who will have a 4-fold or higher response in anti-JE neutralizing antibody titer at 7 days post the booster dose in relation to pre-booster anti-JE neutralizing antibody titer at Year 4.
- Unprimed group consists of participants who will be seronegative for anti-JE IgM at Year 4 prior to the booster dose, who will not have a 4-fold or higher response in anti-JE neutralizing antibody titer at 7 days post the booster dose in relation to pre-booster anti-JE neutralizing antibody titer at Year 4, and who will seroconvert from negative (anti-JE neutralizing antibody titer < 1:10) to positive (anti-JE neutralizing antibody titer ≥ 1:10) between Year 4 prior to the booster dose to 28 days after the booster dose.

GMTs of anti-JE neutralizing antibody along with their 95% CI will be provided for primed group and unprimed group at Year 4 prior to the booster dose, 7 days, and 28 days after the booster dose.

The GMTs at 7 days and 28 days post booster dose will be further compared to the GMTs at Year 4 prior to the booster dose using a paired t-test to evaluate the magnitude of increase in GMTs for primed group and unprimed group. The GMT ratio between 7 days post the booster dose and Year 4 and between 28 days post the booster dose and Year 4 will be provided along with it 95% CI.

7. Safety Analyses

7.1. Safety endpoints

The safety endpoints include:

- Frequency count and proportion of participants reporting immediate reaction occurring within 30 minutes of booster vaccination.
- Frequency count and proportion of participants reporting solicited local and systemic reactions occurring within 7 days of booster vaccination.
- Frequency count and proportion of participants reporting AEs and SAEs occurring within 28 days of booster vaccination.

7.2. Analysis of Safety endpoints

- The number and proportion of participants with at least one occurrence of an immediate reaction (local or systemic reaction, unsolicited AE, and SAE) occurring within 30 minutes of the booster vaccination will be tabulated by study group. The severity, relationship to the booster vaccination, status at 30 minutes (ongoing or not), medication given, 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 safety analysis set. In addition, the immediate reaction will be listed at the 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, change in eating habits, diarrhea, sleepiness, irritability, unusual crying, and vomiting) occurring greater than 30 minutes after receipt of the booster dose through 7 days post vaccination will be summarized by study group along with 95% CI of the percent. 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 the booster dose along with 95% CI of the proportion will be presented by study group. The AEs will be summarized by maximum severity and relationship to the booster vaccination for each study group. The total number, maximum severity, relationship to the booster vaccination, onset date, status/outcome, duration for resolved cases (resolution date – onset date + 1), and medication given of AEs will be also summarized by study group. Summaries will be repeated for AEs found to be related the booster vaccination. A listing of AEs at participant-level will be presented.
- The number and proportion of participants with at least one SAE occurring within 28 days of the booster dose along with 95% CI of the proportion will be presented by study group. The SAEs will be summarized by maximum severity and relationship to the booster vaccination for each study group. The total number, maximum severity, relationship to the booster vaccination, onset date, status/outcome, duration for resolved cases (resolution date – onset date + 1), and medication given of SAEs will be also summarized by study group. Summaries will be repeated for SAEs found to be related the booster vaccination. A listing of SAEs at participant-level will be presented.
- A listing of concomitant medications at participant-level will be provided.

8. Summary of Changes to the Protocol

An interim analysis on immunogenicity assessment (anti-JE neutralizing antibody titer) collected at Visit 01 (Year 3 post primary vaccination) will be conducted in order to gain knowledge on whether or not a booster JE vaccine needs to be given sooner.

Reverse cumulative distribution curves for anti-JE neutralizing antibody titer will be produced at Year 3 and Year 4 post primary vaccination and 7 and 28 post booster vaccination to provide a visual presentation of the comparison of the antibody titers between study groups and how antibody titers change over time.

9. Reference

1. Wilson, E. B. (1927), Probable Inference, the Law of Succession, and Statistical Inference, *Journal of the American Statistical Association*, 22, 209–212.

2. Newcombe, R. G. (1998), Two-sided Confidence Intervals for the Single Proportion: Comparison of Seven Methods, *Statistics in Medicine*, 17, 857–872.

3. Newcombe, R. G. (1998), Interval Estimation for the Difference between Independent Proportions: Comparison of Eleven Methods, *Statistics in Medicine*, 17, 873–890.