Protocol B5091008

A PHASE 3, PLACEBO-CONTROLLED, RANDOMIZED, OBSERVER-BLINDED STUDY TO EVALUATE THE LOT CONSISTENCY, SAFETY, TOLERABILITY, AND IMMUNOGENICITY OF A *CLOSTRIDIUM DIFFICILE* VACCINE IN HEALTHY ADULTS 65 TO 85 YEARS OF AGE

Statistical Analysis Plan
(SAP)

Version: 3.0

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[redacted]

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1. VERSION HISTORY

The statistical analysis plan (SAP) Version 1 for Study B5091008 is based on the protocol dated 08 January 2018. The first SAP amendment (Version 2) is based on protocol amendment 1 dated 08 November 2018. This SAP amendment (Version 3) is updated to align with some changes to the analysis as mentioned in Table 1.

Table 1. Summary of Major Changes in SAP Amendments

SAP Version	Change	Rationale
1	Not Applicable	Not Applicable
CCI	[redacted]	[redacted]
3	Minor updates in Sections 3.4.3, 3.5.1, 3.5.3.1, 4.2, 4.3, 6.1.3, and 6.4.	CCI [redacted] • Adverse events (AEs) will be summarized from day of first dose rather than from signing of the informed consent document (ICD). • Analysis of AEs during the 30 minutes immediately after dosing was deleted because time-of-collection data are not available. • Clarification was added to the duration calculations for reactogenicity events, based on FDA comments for the study data standardization plan. • Immunogenicity analysis using the modified intent-to-treat (mITT) population will be limited based on the relative size of the mITT population. • Clarifications on the populations for analysis of reactogenicity and AEs were added.

2. INTRODUCTION

This SAP provides the detailed methodology for summary and statistical analyses of the data collected in Study B5091008. This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoint definition or its analysis will also be reflected in a protocol amendment. Any major deviations from the methods specified in this document and the protocol will be discussed in the clinical study report.

2.1. Study Objectives

Primary Immunogenicity Objective:

- To demonstrate that the immune responses induced by 3 lots of *Clostridium difficile* vaccine are equivalent, as measured by *C difficile* toxin A– and toxin B–specific neutralizing antibody levels 1 month after the third vaccination when administered in a 3-dose regimen to healthy adults 65 to 85 years of age.

Primary Safety Objective:

- To evaluate the safety of *C difficile* vaccine when administered in a 3-dose regimen to healthy adults 65 to 85 years of age, as measured by the number and percentage of subjects reporting local reactions and systemic events, adverse events (AEs), and serious adverse events (SAEs).

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2.2. Study Design

2.2.1. Description

This is a Phase 3, placebo-controlled, randomized, observer-blinded study to evaluate the lot consistency, safety, tolerability, and immunogenicity of an aluminum hydroxide (AlOH)-containing *C difficile* vaccine (200 µg total toxoid) administered as a 3-dose regimen at Months 0, 1, and 6 in healthy adults 65 to 85 years of age.

Approximately 1316 healthy adults, 65 to 85 years of age, will be randomized into 1 of 4 groups in a 1:1:1:1 ratio (Lot 1: Lot 2: Lot 3: placebo). Subjects withdrawn from the study after randomization will not be replaced, regardless of the reason for withdrawal.

2.2.2. Number of Subjects

In order to meet the immunogenicity primary objective, the study sample size estimate is based upon the evaluation of lot-to-lot consistency on the toxin A– and toxin B–specific neutralizing antibody in terms of geometric mean concentrations (GMCs) 1 month after the third vaccination (Month 7). A 2-fold equivalence margin is used for the study. Assuming the inherent difference for toxin A– and toxin B–specific neutralizing antibody responses is no more than 0.2 (in logarithmic scale) between any 2 lots and the common standard deviations for toxin A– and toxin B–specific neutralizing antibodies are 0.899 and 1.485 (natural log value), respectively (based on the standard deviations for toxin A and toxin B at Month 7 in Study B5091009), a sample size of 263 evaluable subjects per lot will provide an overall 90% power to declare equivalence between all 6 comparisons for both toxin A and toxin B (see Table 2).

Table 2. Power Analysis (Primary Immunogenicity Endpoint)

Criteria	Neutralizing Antibody	Standard Deviation (Log Value)	Assume Observed Log GMC Difference	Number of Evaluable Subjects per Lot	Power[a]	Overall Power (3 Comparisons for Each Toxin)
95% CI for GMC ratio (any 2 lots) is between 0.5 and 2.0	Toxin A	0.899	0.2	263	>99.99%	>99.99%
	Toxin B	1.485	0.2	263	96.72%	90.47%
	Overall power (to meet equivalence for both toxin A and toxin B)					90.47%

Abbreviation: GMC = geometric mean concentration.
a. Using a 2-fold equivalence margin for GMC ratios at a 2-sided, 0.05 alpha level.

Assuming a maximum nonevaluable rate of approximately 20%, a total of 1316 subjects need to be enrolled to meet the primary objective, with a randomization ratio of 1:1:1:1 (Lot 1: Lot 2: Lot 3: placebo).

2.2.3. Schedule of Activities

The schedule of activities table provides an overview of the protocol visits and procedures.

Table 3. Schedule of Activities

Visit ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Visit Description	**Month 0 (Vax 1)**	**Month 1 (Vax 2)**	**Month 2 (Phone Contact)**	**Month 6 (Vax 3)**	**Month 7**
Visit Window (Days)	**1**	**28-42 Days After Visit 1**	**28-42 Days After Visit 2**	**140-168 Days After Visit 2**	**28-42 Days After Visit 4**
Informed consent[a]	X				
Demography[a]	X				
Clinical assessment, including medical history[a]	X				
Record nonstudy vaccinations[b]	X	X	X	X	X
Measure and record height and weight[a]	X				
Oral temperature[a]	X	X		X	
Discuss contraceptive use[b]	X	X	X	X	
Confirm eligibility[b]	X	X	X	X	X
Review temporary delay criteria[a]	X	X		X	
Randomization[a]	X				
Blood draw for immunogenicity assessment[b]	X				X[c]
Vaccination	X	X		X	
Postvaccination observation (at least 30 minutes) and AE assessment	X	X		X	
Issue measuring device and thermometer, and provide instructions on their use, as required	X	X		X	
Assist the subject with downloading the app, or issue provisioned device	X				
Review e-diary completion requirements	X	X		X	

Table 3. Schedule of Activities

Visit ID	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5
Visit Description	**Month 0 (Vax 1)**	**Month 1 (Vax 2)**	**Month 2 (Phone Contact)**	**Month 6 (Vax 3)**	**Month 7**
Visit Window (Days)	**1**	**28-42 Days After Visit 1**	**28-42 Days After Visit 2**	**140-168 Days After Visit 2**	**28-42 Days After Visit 4**
Record AEs/SAEs	X	X	X	X	X
Telephone contact			X		
Review e-diary data[d]	←	—	—	—	→
Collect e-diary					X

Abbreviations: e-diary = electronic diary; Vax = vaccination; ←→ = ongoing/continuous event.

a. Prior to vaccination.
b. Prior to vaccination, if at a vaccination visit.
c. Any AEs occurring up to 48 hours after blood draw must be recorded on the case report form (CRF).
d. E-diary data review is ongoing during subject e-diary data-entry periods (7 days after each vaccination) via an internet-based portal.

3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1. Primary Endpoints

- *C difficile* toxin A– and toxin B–specific neutralizing antibody levels for each lot, expressed as GMCs (neutralization units/mL) at Month 7.

- Local reactions (pain, erythema, and induration), as self-reported in electronic diaries (e-diaries) for up to 7 days following each dose of investigational product.

- Systemic events (fever, vomiting, headache, fatigue, new or worsening muscle pain, and new or worsening joint pain), as self-reported in e-diaries for up to 7 days following each dose of investigational product.

- Nonserious AEs from signing of the informed consent document (ICD) to 1 month after receipt of the third dose of investigational product.

- SAEs from signing of the ICD to 1 month after receipt of the third dose of investigational product.

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3.4. Baseline Variables

3.4.1. Demographics, Physical Examination, and Medical History

Baseline demographic variables for subjects are age at enrollment (signing of the ICD), sex, race, and ethnicity. Age will be calculated as (date of the ICD – date of birth + 1)/365.25 and truncated to the nearest integer less than or equal to the calculated value.

A clinical assessment, including medical history, will be performed on all subjects at Visit 1 to establish a baseline. Significant medical history and observations from any physical examination, if performed, will be documented in the case report form (CRF). Medical history for the subjects will be categorized according to the current version (at the time of reporting) of the Medical Dictionary for Regulatory Activities (MedDRA).

3.4.2. Nonstudy Vaccines and Concomitant Medications

Subjects will be asked to provide a history with the name(s) and date(s) for all vaccinations received from 28 days prior to enrollment until completion of the study. No vaccines other than investigational product should be administered within 28 days before and 28 days after each study vaccination (administered at Visits 1, 2, and 4). Exceptions to this are the seasonal influenza vaccine and pandemic influenza vaccine, which can be given at least 14 days prior to or 14 days after the administration of investigational product. No additional concomitant medications will be recorded.

3.4.3. Baseline Serostatus

An individual subject's baseline serostatus (prior to the first vaccination) will be defined based on the lower limit of quantitation (LLOQ) values for toxin A– and toxin B–specific neutralizing antibody levels as outlined below.

- Toxin A (positive): baseline toxin A–specific neutralizing antibody level ≥ LLOQ for toxin A–specific neutralizing antibody level
- Toxin A (negative): baseline toxin A–specific neutralizing antibody level < LLOQ for toxin A–specific neutralizing antibody level
- Toxin B (positive): baseline toxin B–specific neutralizing antibody level ≥ LLOQ for toxin B–specific neutralizing antibody level

- Toxin B (negative): baseline toxin B–specific neutralizing antibody level < LLOQ for toxin B–specific neutralizing antibody level

In addition, the following 4 groups will also be defined.

- Toxin A (positive)/toxin B (positive): baseline serostatus is positive for both toxin A and toxin B
- Toxin A (positive)/toxin B (negative): baseline serostatus is positive for toxin A and negative for toxin B
- Toxin A (negative)/toxin B (positive): baseline serostatus is negative for toxin A and positive for toxin B
- Toxin A (negative)/toxin B (negative): baseline serostatus is negative for both toxin A and toxin B

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3.5. Safety Endpoints

3.5.1. Adverse Events

Safety endpoints will include Pfizer standard safety endpoints collected in the study. An adverse event (AE) is defined as any untoward medical occurrence and can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease, whether or not related to the subject's participation in the study.

The time period for actively eliciting and collecting AEs for each subject begins from the time the subject provides informed consent through and including Visit 5 (Month 7). For subjects who are screen failures, the active collection period ends when screen failure status is determined.

Overall AEs by category (any AE, related AE, severe AE, serious AE, etc) will be summarized by the pooled lots and the control group from the date of the first dose to 1 month after receipt of the third dose of investigational product. AEs and SAEs will be summarized by the pooled lots and the control group from the date of the first dose to 1 month after receipt of the third dose of investigational product, by MedDRA term. SAEs will also be summarized by the pooled lots and the control group from the date of the first dose throughout the entire study by MedDRA term. In addition, a subject-level listing of all AEs reported will also be provided. In general, all summaries will present the number and percentage of subjects experiencing at least 1 event and the number of events for the pooled lots and the control group, and the exact 2-sided 95% confidence intervals (CIs).

The 3-tier approach will be used for the AE summary. Under this approach, AEs are classified into 1 of 3 tiers. Only for Tier 1 and Tier 2 AEs, the difference in the percentages between the *C difficile* vaccine (pooled lots) group and the placebo group, as well as the 2-sided 95% CI, will be provided. In addition, the p-value will be reported for Tier 1 events. CIs and p-values will be based on the Miettinen and Nurminen method.

Tier 1 events: These are prespecified events of clinical importance and, if any, are maintained in a list in the product's Safety Review Plan.

Tier 2 events: These are events that are not Tier 1 but are "common." A MedDRA preferred term (PT) is defined as a Tier 2 event if its incidence is ≥1.0% in at least 1 vaccine group (combined lots or placebo).

Tier 3 events: These are events that are neither Tier 1 nor Tier 2 events.

3.5.2. Laboratory Data

Serum samples will be obtained for immunogenicity testing at Visit 1 (Month 0, immediately before Dose 1) and Visit 5 (Month 7, 1 month after Dose 3). Both toxin A– and toxin B–specific neutralizing antibody levels will be measured.

3.5.3. Reactogenicity Data

The reactogenicity data collected in the study e-diary will include local reactions (erythema/redness, induration/swelling, and pain at injection site) and systemic events (fever, vomiting, headache, fatigue, new or worsening muscle pain, and new or worsening joint pain). The e-diary will record reactogenicity data from Day 1 to Day 7 starting on the day of each vaccination.

All reactogenicity endpoints will be summarized as proportions of subjects with events, by vaccine group. Additionally, exact 2-sided 95% CIs for proportions will be provided as applicable.

A descriptive summary will be presented for each vaccine lot, the pooled lots, and the control group. For Tier 1 and Tier 2 events, comparison between the pooled lots and the control group will be performed.

Point estimates and the exact 2-sided 95% CIs (using the Clopper-Pearson method) will be provided for proportions of subjects reporting each event.

The proportion of subjects reporting local reactions at the injection site (pain, erythema, and induration), and systemic events (fever, vomiting, headache, fatigue, new or worsening muscle pain, and new or worsening joint pain), on any day within the 7-day period after vaccination will be descriptively summarized by vaccine lot, by pooled lots and control group, and by each vaccination. Severities of local reactions and systemic events reported after each vaccination will also be descriptively summarized by vaccine lot, pooled lots, and control group. Exact 2-sided 95% CIs will be presented with the proportions.

3.5.3.1. Local Reaction Endpoints

Local Reactions: Presence or absence

The presence of redness or swelling is to be recorded in the e-diary as "yes" or "no." If redness or swelling is present, then a second question is to appear requesting the size of the affected area; otherwise, no question is to appear. A measuring device with a scale ranging from 1 to 21 is to be used to measure the largest diameter in whole-number increments. Measurements are to be rounded up to the nearest whole number. If the area is larger than the measuring device can measure, "21+" is to be selected. Measuring device units are converted to centimeters according to the following scale: 1 measuring device unit = 0.5 centimeters.

The presence of redness and swelling is defined according to the following scale:

= ., if missing

= "No," if no or minimal redness or swelling is present, <2.5 cm (<5 measuring device units)

= "Yes," if ≥2.5 cm (≥5 measuring device units)

These categories (., "no," "yes") will be used for derivation of the variables below. For the data summary of the presence ("yes" or "no") of a local reaction, the following variables are required:

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- Presence of each local reaction on any day (Day 1 to Day 7) after each vaccination and after any vaccination.

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- Presence of any local reaction on any day (Day 1 to Day 7) after each vaccination and after any vaccination.

The derivation of CCI "any day" variable is given in Table 4.

Table 4. Derived Variables for Each Local Reaction

Variable	Yes (1)	No (0)	Missing (.)
CCI			
Any day (Day 1 to Day 7)	Subject reports the reaction as "yes" on any day (Day 1 to Day 7)	Subject reports the reaction as "no" on all 7 days or as a combination of "no" and "missing" on all 7 days	Subject did not report on the reaction on any of the 7 days

For any local reaction on any day, a similar rule applies as specified in Table 5.

Table 5. Derived Variables for Any Local Reaction

Variable	Yes (1)	No (0)	Missing (.)
CCI			
Any day (Day 1 to Day 7)	Subject reports any reaction as "yes" on any day (Day 1 to Day 7)	Subject reports all reactions as "no" on all 7 days or as a combination of "no" and "missing" on all 7 days	Subject did not report on any of the reactions on any of the 7 days

Maximum Severity for Local Reactions

Erythema/redness and induration/swelling are measured and recorded in measuring device units (range: 1-21+) and then categorized as absent, mild, moderate, or severe based on the grading scale in Table 6. Pain at the injection site will be assessed by the subjects as mild, moderate, or severe according to the grading scale in Table 6. Only an investigator or a medically qualified person is able to classify a subject's local reaction as Grade 4. Grade 4 could also be determined if a reaction is reported as an SAE.

Table 6. Local Reactions Grading Scale

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain	Does not interfere with activity	Interferes with activity	Prevents daily activity	Emergency room visit or hospitalization for severe pain
Erythema/ redness	2.5 cm to 5.0 cm (5 to 10 measuring device units)	>5.0 cm to 10.0 cm (11 to 20 measuring device units)	>10 cm (≥21 measuring device units)	Necrosis or exfoliative dermatitis
Induration/ swelling	2.5 cm to 5.0 cm (5 to 10 measuring device units)	>5.0 cm to 10.0 cm (11 to 20 measuring device units)	>10 cm (≥21 measuring device units)	Necrosis

The maximum severity (highest grading) of each local reaction within 7 days after each vaccination will be derived. The maximum severity will be derived as follows:

= ., if values are missing for all days (Day 1 to Day 7);

= 0, if the subject reports all reactions as "no" or a combination of "missing" and "no" for all days (Day 1 to Day 7);

= *highest grade* (maximum severity) within 7 days after vaccination, if the answer is "yes" for at least 1 day.

Duration of Each Local Reaction

For subjects experiencing any local reactions (or those with a derived reaction as described in Table 4), the maximum duration (last day of reaction – first day of reaction + 1) will be derived for each study vaccination. Resolution of the event is the last day on which the event is recorded in the e-diary or the date the event ends if it is unresolved during the subject diary-recording period (end date collected on the CRF), unless chronicity is established. If there is no known end date, the duration will be considered unknown and set to "missing." However, if an event is ongoing at the time of a subsequent vaccination, the end date/day for the ongoing event will be the date/day that the next vaccination is administered, which will be used for the duration computation. Subjects with no reported reaction have no duration.

Onset of Each Local Reaction

The onset day of each local reaction and any local reaction will be derived.

The onset day for each local reaction is the first day the subject reports the local reaction, even if the reaction later becomes more severe. The onset day for "any local reaction" will be the first day of any of the 3 local reactions, regardless of severity. Onset day will be "missing" for subjects without the indicated local reaction.

In summary, the following variables will be derived for local reactions:

1. Each local reaction on CCI any day (Day 1 to Day 7) after each vaccination and after any vaccination.
2. Any local reaction on CCI any day (Day 1 to Day 7) after each vaccination and after any vaccination.
3. Maximum severity of each local reaction on any day (Day 1 to Day 7) after each vaccination and after any vaccination.
4. Maximum duration of each local reaction after each vaccination.
5. Onset day of each local reaction after each vaccination.
6. Onset day of any local reaction after each vaccination.

3.5.3.2. Systemic Event Endpoints

Systemic events will be reported via e-diary. Subjects will be asked to assess severity of each event as mild, moderate, or severe as specified in Table 7. Only an investigator is able to classify a subject's systemic event as Grade 4, after physical examination or documentation from another medically qualified source.

Table 7. Systemic Event Grading Scale

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Vomiting	1-2 times in 24 hours	>2 times in 24 hours	Requires IV hydration	Emergency room visit or hospitalization for hypotensive shock
Headache	No interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency room visit or hospitalization for severe headache
Fatigue/ tiredness	No interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency room visit or hospitalization for severe fatigue
New or worsening muscle pain	No interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency room visit or hospitalization for severe new or worsening muscle pain
New or worsening joint pain	No interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency room visit or hospitalization for severe new or worsening joint pain

Abbreviation: IV = intravenous.

For each systemic event, the following variables will be available, similar to local reactions:

1. Each systemic event on CCI [redacted] any day (Day 1 to Day 7) after each vaccination and after any vaccination.
2. Any systemic event (including fever as described in Section 3.5.3.3) on CCI [redacted] any day (Day 1 to Day 7) after each vaccination and after any vaccination.
3. Maximum severity of each systemic event on any day (Day 1 to Day 7) after each vaccination and after any vaccination.
4. Maximum duration of each systemic event after each vaccination.
5. Onset day of each systemic event after each vaccination.
6. Onset day of any systemic event after each vaccination.

The derivation of these variables is similar to the derivation of the variables for local reactions (Section 3.5.3.1).

3.5.3.3. Temperature

Oral temperature will be collected in the e-diary, in the evening, daily for 7 days after vaccination. It will also be collected at any time during the e-diary data collection periods that fever is suspected. The highest temperature for each day will be recorded in the e-diary. The protocol defines fever as an oral temperature ≥38.0°C (≥100.4°F). Fever will be categorized as specified in Table 8.

Table 8. Scale for Fever

Mild (Grade 1)	38.0-38.4°C (100.4-101.1°F)
Moderate (Grade 2)	38.5-38.9°C (101.2-102.0°F)
Severe (Grade 3)	39.0-40.0°C (102.1-104.0°F)
Potentially life threatening (Grade 4)	>40.0°C (>104.0°F)

Similar to the derivations of systemic events and local reactions, fever will be derived for:

1. Fever on CCI any day (Day 1 to Day 7) after each vaccination and after any vaccination.

2. Highest fever (maximum severity) on any day (Day 1 to Day 7) after each vaccination and after any vaccination.

3. Maximum duration of fever after each vaccination.

4. Onset day of fever after each vaccination.

Temperatures <35.0°C and >42.0°C will be excluded from the analysis.

3.5.4. E-Diary Completion

For any given day, an e-diary will be transmitted and considered as complete if all expected data (the 3 local reactions and the 6 systemic events, including fever) are available. If all data are missing for all items on the e-diary, for all days following vaccination, the e-diary will be considered not transmitted. An e-diary will be considered completed if all expected data for all days are available (ie, not missing) and data are valid. Otherwise, the e-diary will be considered incomplete.

For e-diaries, an indicator variable for the percentage of days without data will be derived as follows:

= 1, if data have been transmitted and are complete for 7 days (100%)

= 2, if data have been transmitted and are complete for 6 days (≥75% to <100%)

= 3, if data have been transmitted and are complete for 4 or 5 days (≥50% to <75%)

= 4, if data have been transmitted and are complete for 2 or 3 days (≥25% to <50%)

= 5, if data have been transmitted and are complete for 0 to 1 day (<25%)

4. ANALYSIS SETS

Data for all subjects will be assessed to determine if subjects meet the criteria for inclusion in each analysis population prior to unblinding and releasing the database, and classifications will be documented per standard operating procedures.

4.1. Evaluable Immunogenicity Population

The evaluable immunogenicity (EI) population will be the primary population for the immunogenicity analyses. The EI population will consist of all study subjects who:

- Have been enrolled in the study and receive all 3 doses of investigational product to which they were randomized,
- Have blood drawn for assay testing within 23 to 47 days after Visit 4,
- Have valid and determinate assay results for either toxin A or toxin B for the specified analysis, and
- Have no major protocol deviations

4.2. Modified Intent-to-Treat Population

The modified intent-to-treat (mITT) population will include all randomized subjects who have received at least 1 dose of the investigational product and have at least 1 valid and determinate assay result for either toxin A or toxin B for the specified analysis.

Immunogenicity analyses using the mITT population will be conducted only if the mITT population size exceeds the size of the EI population by >5%. If the difference between the sizes of the mITT and EI populations is ≤5%, only the analyses based on the EI population will be provided.

4.3. Safety Analysis Population

The safety analysis (SAF) population will include all subjects who receive at least 1 dose of an investigational product. For reactogenicity analyses by dose/lot, subjects who received a different dose/lot from the dose/lot they were assigned will be included in the SAF population for the summaries of individual vaccinations up until the point their dose/lot differs from the assigned dose/lot, at which point they will no longer be included. For AE analysis, such subjects will be included in the AE summary in the group according to the first vaccine dose/lot received.

4.4. Other Analysis Sets

No other analysis set will be defined for the study.

5. GENERAL METHODOLOGY AND CONVENTIONS

The primary objective of lot-to-lot consistency will be evaluated by GMC ratios, along with 95% CIs, for each *C difficile* toxin A– or toxin B–specific neutralizing antibody level determined at 1 month after Dose 3 using subjects in the EI population.

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[redacted]

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The *C difficile* toxin A– and toxin B–specific neutralizing antibody levels at each blood sampling time point will be summarized by GMCs and the associated 95% CIs. Any *C difficile* toxin A– or toxin B–specific neutralizing antibody level that is below the LLOQ will be assigned a value of 0.5 × LLOQ. GMCs and the associated 2-sided 95% CIs will be derived by calculating means and CIs on the natural log scale based on the t-distribution, and then exponentiating the results.

5.1. Hypotheses and Decision Rules

5.1.1. Analysis of the Primary Immunogenicity Endpoint

The primary objective of lot consistency will be tested for each *C difficile* toxin A– or toxin B–specific neutralizing antibody level determined at 1 month after Dose 3 using subjects in the EI population. The null hypothesis (H_0) for the lot consistency is:

$$H_0 : \left|\ln(\mu_1)-\ln(\mu_2)\right| \geq \ln(2) \text{ OR } \left|\ln(\mu_1)-\ln(\mu_3)\right| \geq \ln(2) \text{ OR } \left|\ln(\mu_2)-\ln(\mu_3)\right| \geq \ln(2)$$

where $\ln(\mu_1)$, $\ln(\mu_2)$, and $\ln(\mu_3)$ are the means of the natural logarithm-transformed data of the *C difficile* toxin A– or toxin B–specific neutralizing antibody levels from subjects receiving *C difficile* vaccine from Lots 1, 2, and 3, respectively, measured 1 month after the third vaccination (Month 7) with *C difficile* vaccine. The neutralizing antibody–level data will be logarithmically transformed for analysis. GMC ratios, along with 2-sided 95% CIs, will be computed for each toxin A– or toxin B–specific neutralizing antibody concentration for each pair of lots (a total of 6 comparisons). The primary immunogenicity objective of the lot-to-lot consistency will be achieved if the 2-sided 95% CIs for the GMC ratios for all 6 comparisons are within the interval (0.5, 2), for both *C difficile* toxin A and toxin B.

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[redacted]

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[redacted]

5.2. General Methods

Unless otherwise explicitly stated, descriptive statistics for continuous variables are n, mean, median, standard deviation, minimum, and maximum. Descriptive statistics for categorical variables are n, percentage, and total (N). There is no screening period in this study; Day 1 (Visit 1) is considered as the date the subject receives their first vaccination.

5.2.1. Analyses for Binary Data

Exact 2-sided 95% CIs will be computed using the Clopper-Pearson method for any proportion of subjects with *C difficile* toxin A– or toxin B–specific neutralizing antibody level ≥ cutoff (LLOQ).

If r equals the number of responses and n equals the number of subjects, then it follows that $p = r/n$ is the estimate of the proportion of responses. An exact 95% CI (or Clopper-Pearson confidence limit) can be computed by solving the following 2 equations. For the lower limit (P_L) and the upper limit (P_U), use:

$$P_L = \frac{rF_L}{(rF_L+(n-r+1))} \quad and \quad P_U = \frac{(r+1)F_U}{(n-r)+(r+1)F_U}$$

where F_L is the quantile from the F distribution for α=0.025, with numerator degrees of freedom equal to $2r$ and denominator degrees of freedom equal to $2(n-r+1)$. F_U is the quantile from the F distribution for α=0.975, with numerator degrees of freedom equal to $2(r+1)$ and denominator degrees of freedom equal to $2(n-r)$. When r equals 0, F_L is set to 1.0 so P_L equals 0. When r equals n, F_U is set to 1.0 so P_U equals 1. The CI using the F distribution is described by Collett (1991).[1]

For Tier 1 and Tier 2 AEs, the difference in the percentages between *C difficile* vaccine pooled lots and placebo will be provided. The Miettinen and Nurminen (1985)[2] method will be used to derive the 95% CI for the risk difference between vaccine combined active arms and placebo. The p-value from the Miettinen and Nurminen method will also be provided for Tier 1 events.

5.2.2. Analyses for Continuous Data

The *C difficile* toxin A– or toxin B–specific neutralizing antibody levels at each blood sampling time point will be summarized by GMCs and the associated 95% CIs for each vaccine group and also for combined active arms. The GMC will be calculated as the mean of the assay results after making the logarithm transformation and then exponentiating the result. Two-sided 95% CIs will be constructed by obtaining the CI for the mean of the logarithmically transformed assay results based on the t-distribution, and then exponentiating the limits.

5.3. Methods to Manage Missing Data

5.3.1. Safety Data

Standard algorithms for handling missing AE dates and missing AE severity will be applied following the safety rulebook summary developed by Pfizer.

5.3.1.1. Reactogenicity Data

For derived variables based on reactogenicity data, if any day of the 7-day e-diary is available, the "any day (Day 1 to Day 7)" data will be considered as nonmissing. Subjects are excluded from the analysis if they do not receive the particular dose or the safety data are missing on all days within the interval.

The reactogenicity data are collected through the e-diary, which does not allow subjects to skip the question. Therefore, for a specific day, as long as the e-diary data are transferred for that day, all of the reactogenicity data for the subject on that day are nonmissing. The e-diary transmission and completion status will be summarized per Section 3.5.4. The e-diary completion summary will provide the missing information for the reactogenicity data.

6. ANALYSES AND SUMMARIES

The primary objective is to establish lot-to-lot consistency on the *C difficile* toxin A– and toxin B–specific neutralizing antibody levels in terms of GMCs, 1 month after the third vaccination (Month 7).

6.1. Primary Endpoints

6.1.1. *C difficile* Toxin A– and Toxin B–Specific Neutralizing Antibody Levels

Endpoint: *C difficile* toxin A–specific neutralizing antibody levels for each lot, expressed as GMCs

- Analysis time points: Visit 5 (1 month after the third vaccination)

- Analysis population: EI population
- Analysis methodology: GMC ratios and the 95% CIs
- Supporting objective: primary immunogenicity objective

Reporting results:

- Two-sided 95% CIs for the GMC ratios for all 3 comparisons

Endpoint: *C difficile* toxin B–specific neutralizing antibody levels for each lot, expressed as GMCs

- Analysis time points: Visit 5 (1 month after the third vaccination)
- Analysis population: EI population
- Analysis methodology: GMC ratios and the 95% CIs
- Supporting objective: primary immunogenicity objective

Reporting results:

- Two-sided 95% CIs for the GMC ratios for all 3 comparisons

6.1.2. Local Reactions and Systemic Events as Self-Reported in E-Diaries

Endpoints: (1) Local reactions (pain, erythema, and induration); (2) maximum severity as self-reported in the e-diary for up to 7 days following vaccination at Visit 1, Visit 2, and Visit 4

- Analysis time points: occurring on CCI any day during Day 1 to Day 7 following vaccination at Visit 1, Visit 2, and Visit 4
- Analysis population: SAF population
- Analysis methodology: point estimates and Clopper-Pearson CIs
- Supporting objective: primary safety objective

Reporting results:

- The number and percentage of subjects reporting: (1) a reaction; (2) maximum severity, on CCI any day during Day 1 to Day 7, and the associated 95% CIs

Endpoints: (1) Systemic events (fever, vomiting, headache, fatigue, new or worsening muscle pain, and new or worsening joint pain); (2) maximum severity as self-reported in the e-diary for up to 7 days following vaccination at Visit 1, Visit 2, and Visit 4

- Analysis time points: occurring on CCI any day during Day 1 to Day 7 following vaccination at Visit 1, Visit 2, and Visit 4
- Analysis population: SAF population
- Analysis methodology: point estimates and Clopper-Pearson CIs
- Supporting objective: primary safety objective

Reporting results:

- The number and percentage of subjects reporting: (1) a systemic event; (2) maximum severity, on CCI any day during Day 1 to Day 7, and the associated 95% CIs

6.1.3. Adverse Events

Endpoint: AEs from the date of the first dose to Visit 5 (Month 7)

- Analysis time points: Visit 1 to Visit 5
- Analysis population: SAF population
- Analysis methodology: point estimates and Clopper-Pearson CIs
- Supporting objective: primary safety objective

Reporting results:

- The number and percentage of subjects reporting AEs from Visit 1 to Visit 5 and the associated 95% CIs

Endpoint: SAEs from the date of the first dose to Visit 5

- Analysis time points: Visit 1 to Visit 5
- Analysis population: SAF population
- Analysis methodology: Clopper-Pearson CIs
- Supporting objective: primary safety objective

Reporting results:

- The number and percentage of subjects reporting SAEs from Visit 1 to Visit 5 and the associated 95% CIs

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6.4. Baseline and Other Summaries and Analyses

6.4.1. Demographics and Medical History

Descriptive summary statistics of baseline demographics and medical history as described in Section 3.4 will be provided.

6.4.2. Study Conduct and Subject Disposition

The numbers and percentages of subjects who signed the ICD, withdrew during the study period, and completed the study will be included in a disposition summary. The reasons for withdrawal will also be tabulated. The reasons for withdrawal will be those specified in the database; no rewording/recoding will be done.

6.4.3. Concomitant Medications and Nondrug Treatments

A summary table will be provided for any vaccinations given to the subject for the duration of the study. Concomitant medications used to treat SAEs will be provided in an AE listing.

6.5. Illness Visit Outcomes, Laboratory Results, and Safety Summaries and Analyses

All data summaries for illness visit outcomes, laboratory results, and AEs will be provided.

All safety analyses for AEs will be summarized in accordance with the Pfizer reporting standard, including all subjects enrolled in the study.

7. INTERIM ANALYSES

No interim analysis is planned for the study. The final analysis will be performed after all the safety and serology data are available.

8. REFERENCES

1. Collett D. Modelling binary data. 1st ed. London, England: Chapman & Hall; 1991.

2. Miettinen O, Nurminen M. Comparative analysis of two rates. Stat Med 1985;4(2):213-26.