Protocol B5091009

A PHASE 2, PLACEBO-CONTROLLED, RANDOMIZED, OBSERVER-BLINDED STUDY TO EVALUATE THE SAFETY, TOLERABILITY, AND IMMUNOGENICITY OF TWO 3-DOSE REGIMENS OF A *CLOSTRIDIUM DIFFICILE* VACCINE IN HEALTHY ADULTS AGED 65 TO 85 YEARS

Statistical Analysis Plan
(SAP)

Version: 2.0

Date: 17-June-2016

TABLE OF CONTENTS

LIST OF TABLES

1. VERSION HISTORY

The statistical analysis plan (SAP) Version 1 for Study B5091009 was based on the protocol dated 01 April 2015. This SAP amendment is based on protocol amendment 1 dated 11 April 2016.

Table 1. Summary of Major Changes in SAP Amendments

SAP Version	Change	Rationale
1.0	Not applicable	Not applicable
2.0	Major changes include adding objectives, endpoints, and analysis for the extension stage of the study and an additional interim analysis discussed in protocol amendment 1	Protocol amendment 1, 11 April 2016

2. INTRODUCTION

This SAP provides the detailed methodology for summary and statistical analyses of the data collected in Study B5091009. 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.

2.1. Study Objectives

2.1.1. Primary Immunogenicity Objectives (PI)

- To describe the immunogenicity of 2 antigen dose levels (100 µg and 200 µg total toxoid) of *Clostridium difficile* vaccine when administered as a 3-dose regimen (Days 1, 8, and 30) to healthy adults aged 65 to 85 years, as measured by *C difficile* toxin A– and toxin B–specific neutralizing antibody levels at Day 37 (7 days after Dose 3).

- To describe the immunogenicity of 2 antigen dose levels (100 µg and 200 µg total toxoid) of *C difficile* vaccine when administered as a 3-dose regimen (Months 0, 1, and 6) to healthy adults aged 65 to 85 years, as measured by *C difficile* toxin A– and toxin B–specific neutralizing antibody levels at Month 7 (1 month after Dose 3).

2.1.2. Primary Safety Objective (PS)

- To assess the safety and tolerability of 2 antigen dose levels (100 µg and 200 µg total toxoid) of *C difficile* vaccine when administered as a 3-dose regimen (either Days 1, 8, and 30 or Months 0, 1, and 6) to healthy adults aged 65 to 85 years, by measuring local reactions and systemic events reported on subjects' electronic diaries (e-diaries), adverse events (AEs), and serious AEs (SAEs).

2.1.3. Secondary Immunogenicity Objectives (SI)

- To describe the immunogenicity of 2 antigen dose levels (100 µg and 200 µg total toxoid) of *C difficile* vaccine when administered in a 3-dose regimen (either Days 1, 8, and 30 or Months 0, 1, and 6) to healthy adults aged 65 to 85 years, as measured by *C difficile* toxin A– and toxin B–specific neutralizing antibody levels at multiple time points following vaccination.

- To describe the kinetics of the immune response in healthy adults aged 65 to 85 years for up to 12 months following the administration of 3 doses of *C difficile* vaccine.

- To describe the immunogenicity of a fourth dose of *C difficile* vaccine as measured by *C difficile* toxin A– and toxin B–specific neutralizing antibody levels at multiple time points following vaccination.

- To describe the kinetics of the immune response in healthy adults aged 65 to 85 years for up to 36 months following the administration of a fourth dose of *C difficile* vaccine.

2.1.4. Secondary Safety Objective (SS)

- To assess the safety and tolerability of a fourth dose of *C difficile* vaccine by measuring local reactions and systemic events reported on subjects' e-diaries, AEs, and SAEs.

2.1.5. CCI (C)

- CCI
- [redacted]
- [redacted]

2.2. Study Design

This is a Phase 2, placebo-controlled, randomized, observer-blinded study to assess the safety, tolerability, and immunogenicity of 2 antigen dose levels (100 µg and 200 µg total toxoid) of aluminum hydroxide–containing *C difficile* vaccine administered as a 3-dose regimen: either at Days 1, 8, and 30 or at Months 0, 1, and 6.

Subjects will be assigned to 1 of the 2 dosing regimens (Days 1, 8, and 30 or Months 0, 1, and 6) and then randomly assigned in parallel in a 3:3:1 ratio to receive *C difficile* vaccine (100 µg or 200 µg total toxoid) or placebo (saline).

Subjects in both dosing regimens who received the first 3 doses of *C difficile* vaccine (100 µg or 200 µg) will be enrolled into an extension stage. These subjects will receive a fourth dose of either *C difficile* vaccine at the same antigen dose level (100 µg or 200 µg) as they received previously or placebo, approximately 1 year after their third dose. These subjects will be followed for a further 3 years (visits approximately every 6 months) to assess antibody persistence. Subjects originally randomized to placebo in either dosing regimen will not continue into the extension stage.

Approximately 854 healthy adults aged 65 to 85 years will be enrolled at approximately 15 sites in the United States. Subjects withdrawn from the study after randomization will not be replaced, regardless of the reason for withdrawal.

For each dosing regimen, approximately 366 subjects will receive the *C difficile* vaccine (at either 100 or 200 µg total toxoid dose level) and approximately 61 subjects will receive placebo (saline).

Table 2. Vaccine Groups and Number of Subjects per Group and per Dose Regimen

Vaccine Group	Vaccine Formulation Description	Dosing Regimen	Number of Subjects
1[a]	Aluminum hydroxide–containing *C difficile* vaccine (100-µg antigen dose)	Days 1,8,30	183
2[a]	Aluminum hydroxide–containing *C difficile* vaccine (200-µg antigen dose)	Days 1,8,30	183
3	Placebo (saline)	Days 1,8,30	61
4[a]	Aluminum hydroxide–containing *C difficile* vaccine (100-µg antigen dose)	Months 0,1,6	183
5[a]	Aluminum hydroxide–containing *C difficile* vaccine (200-µg antigen dose)	Months 0,1,6	183
6	Placebo (saline)	Months 0,1,6	61
Total			**854**

a. Subjects in these groups will be asked to enter the extension stage

All eligible subjects who received 3 doses of *C difficile* vaccine (Groups 1, 2, 4, and 5) will be asked to enter the extension stage. Subjects enrolled in the extension stage will be rerandomized to receive a fourth dose of either *C difficile* vaccine at the same antigen dose level (100 µg or 200 µg) as they received previously or placebo.

3. ENDPOINTS AND BASELINE VARIABLES: DEFINITIONS AND CONVENTIONS

3.1. Primary Endpoints

3.1.1. Primary Immunogenicity Endpoints

At Day 37 (7 days after Dose 3 for subjects receiving the Day 1, 8, and 30 regimen) and at Month 7 (1 month after Dose 3 for subjects receiving the Month 0, 1, and 6 regimen), the proportions of subjects in each vaccine group with:

- Toxin A–specific neutralizing antibody level (neutralization units/mL) ≥ the specified threshold for toxin A;

- Toxin B–specific neutralizing antibody level (neutralization units/mL) ≥ the specified threshold for toxin B; and

- Both toxin A– and toxin B–specific neutralizing antibody levels (neutralization units/mL) ≥ the specified threshold for toxin A and the specified threshold for toxin B, respectively.

CCI [redacted]

3.1.2. Primary Safety Endpoints

- Numbers and proportions of subjects reporting local reactions (pain, erythema, and induration) and their severity, as self-reported on e-diaries for up to 14 days following Vaccinations 1, 2, and 3.

- Numbers and proportions of subjects reporting systemic events (fever, vomiting, diarrhea, headache, fatigue, new or worsening muscle pain, and new or worsening joint pain) and their severity, as self-reported on e-diaries for up to 14 days following Vaccinations 1, 2, and 3.

- Numbers and proportions of subjects reporting AEs from the first vaccination up to 28 days after the third vaccination, categorized according to the Medical Dictionary for Regulatory Activities (MedDRA).

- Numbers and proportions of subjects reporting SAEs from the first vaccination until 6 months after the third vaccination, categorized according to MedDRA.

3.2. Secondary Endpoints

3.2.1. Secondary Immunogenicity Endpoints

At Day 37 (7 days after Dose 3 for subjects receiving the Day 1, 8, and 30 regimen) and at Month 7 (1 month after Dose 3 for subjects receiving the Month 0, 1, and 6 regimen):

- Toxin A– and toxin B–specific neutralizing antibody levels, expressed as geometric mean concentrations (GMCs) (neutralization units/mL).

- Geometric mean fold rises (GMFRs) from baseline (before Dose 1) in:

  - Toxin A–specific; and

  - Toxin B–specific neutralizing antibody levels (neutralization units/mL).

- Proportions of subjects in each vaccine group with ≥4-fold, ≥8-fold, ≥16-fold, and ≥32-fold rises from baseline in:
  - Toxin A–specific;
  - Toxin B–specific; and
  - Both toxin A– and toxin B–specific neutralizing antibody levels (neutralization units/mL).

For subjects receiving the Day 1, 8, and 30 regimen, on Day 1 (immediately before Dose 1), Day 8 (immediately before Dose 2), Day 15 (7 days after Dose 2), Day 30 (immediately before Dose 3), and Months 2 (1 month after Dose 3), 4 (3 months after Dose 3), 7 (6 months after Dose 3), and 13 (12 months after Dose 3); or for subjects receiving the Month 0, 1, and 6 regimen, on Day 1 (immediately before Dose 1), Day 30 (immediately before Dose 2), Day 37 (7 days after Dose 2), Months 2 (1 month after Dose 2) and 6 (immediately before Dose 3), Day 187 (7 days after Dose 3), and Months 12 (6 months after Dose 3) and 18 (12 months after Dose 3):

- Proportions of subjects in each vaccine group with:
  - Toxin A–specific neutralizing antibody level (neutralization units/mL) ≥ the specified threshold for toxin A;
  - Toxin B–specific neutralizing antibody level (neutralization units/mL) ≥ the specified threshold for toxin B; and
  - Both toxin A– and toxin B–specific neutralizing antibody levels (neutralization units/mL) ≥ the specified threshold for toxin A and the specified threshold for toxin B, respectively (these parameters will also be assessed at baseline).

CCI [redacted]

- Toxin A– and toxin B–specific neutralizing antibody levels, expressed as GMCs (neutralization units/mL).
- GMFRs from baseline in:
  - Toxin A–specific; and
  - Toxin B–specific neutralizing antibody levels (neutralization units/mL).
- Proportions of subjects in each vaccine group with ≥4-fold, ≥8-fold, ≥16-fold, and ≥32-fold rises from baseline in:

- Toxin A–specific;
- Toxin B–specific; and
- Both toxin A– and toxin B–specific neutralizing antibody levels (neutralization units/mL).

Baseline in the above endpoints is the associated last measurement prior to the first vaccination on Day 1.

For subjects enrolled in the extension stage; at the fourth dose (immediately before the fourth dose), at Days 8 and 30 after the fourth dose, and at Months 6, 12, 18, 24, 30, and 36 after the fourth dose:

- Proportions of subjects in each vaccine group with
  - Toxin A–specific neutralizing antibody level (neutralization units/mL) ≥ the specified threshold for toxin A;
  - Toxin B–specific neutralizing antibody level (neutralization units/mL) ≥ the specified threshold for toxin B; and
  - Both toxin A– and toxin B–specific neutralizing antibody levels (neutralization units/mL) ≥ the specified threshold for toxin A and the specified threshold for toxin B, respectively (these parameters will also be assessed at baseline).
- Toxin A– and toxin B–specific neutralizing antibody levels, expressed as GMCs (neutralization units/mL).
- GMFRs from baseline in
  - Toxin A–specific; and
  - Toxin B–specific neutralizing antibody levels (neutralization units/mL).
- Proportions of subjects in each vaccine group with ≥4-fold, ≥8-fold, ≥16-fold, and ≥32-fold rises from baseline in
  - Toxin A–specific;
  - Toxin B–specific; and
  - Both toxin A– and toxin B–specific neutralizing antibody levels (neutralization units/mL).

3.2.2. Secondary Safety Endpoints

- Numbers and proportions of subjects reporting local reactions (pain, erythema, and induration) and their severity, as self-reported on e-diaries for to 14 days following the fourth vaccination.

- Numbers and proportions of subjects reporting systemic events (fever, vomiting, diarrhea, headache, fatigue, new or worsening muscle pain, and new or worsening joint pain) and their severity, as self-reported on e-diaries for 14 days following the fourth vaccination.

- Numbers and proportions of subjects reporting AEs from the time of the fourth vaccination up to 28 days after the fourth vaccination categorized according to MedDRA.

- Numbers and proportions of subjects reporting SAEs from the time of the fourth vaccination until 6 months after the fourth dose of vaccine, categorized according to MedDRA.

3.3. CCI

- CCI

3.4. CCI

CCI

- CCI [redacted]
- [redacted]

[redacted]

3.5. Safety Endpoints

3.5.1. Adverse Events

A 3-tier approach will be used to summarize AEs. Under this approach, AEs are classified into 1 of 3 tiers. Different analyses will be performed for different tiers (see Section 6.5.1).

Tier 1 events: These are prespecified events of clinical importance and, if any, will be 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 there are at least 5% in any vaccine group.

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

AEs will be captured and reported in accordance with Pfizer reporting standards.

AEs occurring prior to the first vaccination will be excluded from AE analysis, unless the severity increases after dosing. AEs occurring between the informed consent document (ICD) signoff and prior to the first vaccination will be summarized separately.

3.5.2. CCI [redacted]

- CCI [redacted]

3.5.3. Reactogenicity Endpoints

Reactogenicity data captured in the e-diary consist of local reactions (erythema/redness, induration/swelling, and pain at the injection site), systemic events (fever, vomiting, diarrhea, headache, fatigue, new or worsening muscle pain, and new or worsening joint pain), and use of antipyretics/pain medication to treat symptoms.

3.5.3.1. Local Reactions

Local reactions reported in the e-diary are erythema/redness, induration/swelling, and pain.

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 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 for the originally planned stage [first 3 vaccinations] and the extension stage [fourth vaccination] are required:

- Presence ("Yes" or "No") of each local reaction on each day (Day 1 to Day 7 after the first vaccination on the Day 1, 8, and 30 regimen, and Day 1 to Day 14 after all other vaccinations in both regimens).
- Presence ("Yes" or "No") of each local reaction on any day (Day 1 to Day 7 after the first vaccination on the Day 1, 8, and 30 regimen, and Day 1 to Day 14 after all other vaccinations in both regimens).
- Presence ("Yes" or "No") of any local reactions on any day (Day 1 to Day 7 after the first vaccination on the Day 1, 8 and 30 regimen, and Day 1 to Day 14 after all other vaccinations in both regimens).
- Presence ("Yes" or "No") of each local reaction with severe or greater severity (Grade 3 or Grade 4, as defined in Table 5) on each day (Day 1 to Day 7 after the first vaccination on the Day 1, 8, and 30 regimen, and Day 1 to Day 14 after all other vaccinations in both regimens).

Table 3 explains the algorithm to derive the presence of a reaction ("Yes" or "No") during a given time period.

Table 3. Derived Variables for Each Local Reaction Within a Specified Time Interval

Variable[a]	Yes (1)	No (0)	Missing (.)
Any day (Days 1-X)	Subject reports the reaction as "Yes" on any day from Days 1-X	Subject reports the reaction as "No" on all X days or as a combination of "No" and missing on all X days	Subject reports the reaction as missing on all X days

a. The variable will be derived for each of the 3 local reactions (pain, erythema/redness, and induration/swelling), with the time interval Day 1 to Day X after each vaccination. X = 7 days after the first vaccination on the Day 1, 8, and 30 regimen, and X = 14 days after all other vaccinations in both regimens.

For "any local reaction on any day," a similar rule applies, as specified below in Table 4.

Table 4. Derived Variables for Any Local Reaction Within a Specified Time Interval

Variable[a]	Yes (1)	No (0)	Missing (.)
Any day (Days 1-X)	Subject reports any local reaction as "Yes" on any day from Days 1-X	Subject reports the reaction as "No" on all X days or as a combination of "No" and missing on all X days on all 3 local reactions	Subject reports all of the local reactions as missing on all X days

a. The variable will be derived for Day 1 to Day X after each vaccination. X = 7 days after the first vaccination on the Day 1, 8 and 30 regimen, and X = 14 after all other vaccinations in both regimens.

In addition, each local reaction and any local reaction on any day (Days 1- X) after **any** vaccination in the originally planned phase will be derived, where X = 7 days after the first vaccination on the Day 1, 8 and 30 regimen, and X = 14 days after the second and, third vaccinations on the Day 1, 8, and 30 regimen and after each vaccination on the Month 0, 1, and 6 regimen.

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 5. Pain at the injection site will be assessed by the subject as absent, mild, moderate, or severe according to the grading scale in Table 5.

For each local reaction, the maximum severity grade of the reaction will be derived for the e-diary collection period (Day 1 to Day 7 after the first vaccination on the Day 1, 8, and 30 regimen, Day 1 to Day 14 after the second and third vaccinations on the Day 1, 8, and 30 regimen, and Day 1 to Day 14 after each vaccination on the Month 0, 1, and 6 regimen) and

after the fourth vaccination of the extension stage in both regimens. The severity grading scale of the local reaction is presented in Table 5.

The maximum severity will be derived as follows:

= missing, if all values are missing for all days (Day 1 to Day 7 after the first vaccination on the Day 1, 8, and 30 regimen, and Day 1 to Day 14 after all other vaccinations in both regimens).

= 0, if the subject reports all reactions as "No", or a combination of missing and "No" for all days (Day 1 to Day 7 after the first vaccination on the Day 1, 8, and 30 regimen, and Day 1 to Day 14 after all other vaccinations in both regimens).

= highest grade (maximum severity) within 7 days of vaccination after the first vaccination on the Day 1, 8, and 30 regimen, and within 14 days after all other vaccinations in both regimens, if the answer is not "No" for at least 1 day.

Only an investigator or 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.

Maximum severity of each local reaction on any day (Days 1- X) after **any** vaccination in the originally planned phase will also be derived, where X = 7 days after the first vaccination on the Day 1, 8 and 30 regimen, and X = 14 after the second, third vaccinations on the Day 1, 8, and 30 regimen and after each vaccination on the Month 0, 1, and 6 regimen.

Table 5. Local Reaction Grading Scale

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Grade 4
Pain	Does not interfere with activity	Interferes with activity	Prevents daily activity	Emergency room visit or hospitalization
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

Duration of Each Local Reaction

The duration of each local reaction will be calculated as the number of days from the start of the first reported reaction to the resolution of the last reported reaction, inclusive. Resolution is the last day on which the reaction is recorded in the e-diary if the reaction lasted 7 days or less for the first vaccination on the Day 1, 8, and 30 regimen or 14 days or less for the second and third vaccinations on the Day 1, 8, and 30 regimen, or 14 days or less for each

vaccination on the Month 0, 1, and 6 regimen, and fourth vaccination of the extension stage in both regimens, or the date the reaction ended if it continues beyond Day 7 for the first vaccination on the Day 1, 8, and 30 regimen and Day 14 for the second and third on the Day 1, 8, and 30 regimen and for each vaccination on the Month 0, 1, and 6 regimen, and fourth vaccination of the extension stage in both regimens, (the latter will be collected on the case report form [CRF]). If there is no known date when the reaction ends, then duration will be missing (unknown). Subjects with no reported reaction have no duration, because it is not applicable. However, the date on which a reaction ended for one vaccination should not be after the beginning of the next vaccination. If a reaction or an event is ongoing at the time of a subsequent vaccination, the duration will be set to unknown.

Onset of Local Reactions

The onset day of each local reaction and any local reaction since the last vaccination will be derived.

For the onset day of each local reaction, if a subject reports a change in severity of the local reaction, the first day of reporting that specific local reaction will be used in the calculation.

For the onset day of any local reaction, the first day of reporting any severity of any local reaction will be used in the calculation.

Maximum Diameter for Erythema/Redness and Induration/Swelling

The diameter of the affected area for erythema/redness and induration/swelling within 7 days after the first vaccination on the Day 1, 8, and 30 regimen and within 14 days after the second, third vaccinations on the Day 1, 8, and 30 regimen and after each vaccination on the Month 0, 1, and 6 regimen, and fourth vaccination in their extension stage of both regimens will be derived following each vaccination. The measuring device units are converted to centimeters for the analyses (1 measuring device unit = 0.5 centimeters).

3.5.3.2. Systemic Events

The systemic events reported in the e-diary are fever, vomiting, diarrhea, headache, fatigue, new or worsening muscle pain, and new or worsening joint pain. Fever is defined as an oral temperature of ≥38.0°C (100.4°F). The highest temperature for each day will be recorded in the e-diary. Temperature will be collected daily in the e-diary from Day 1 to Day 7 for the first vaccination on the Day 1, 8, and 30 regimen and Day 1 to Day 14 for the second and third vaccinations on the Day 1, 8, and 30 regimen and after each vaccination on the Month 0, 1, and 6 regimen, and fourth vaccination in their extension stage until fever has resolved (1 day of temperature less than 38.0°C [100.4°F]). For ongoing fever on Day 7 for the first vaccination on the Day 1, 8, and 30 regimen or Day 14 for the second and third vaccinations on the Day 1, 8, and 30 regimen and after each vaccination on the Month 0, 1, and 6 regimen, and fourth vaccination in their extension stage of both regimens, the stop date will be recorded in the CRF. For any other systemic event reported as present, the subjects will also note the severity of the event.

Table 6 shows the details for the severity grading scales of each systemic event.

Any temperature recorded as <95.0°F (35.0°C) and >107.6°F (42.0°C) will be considered as invalid data and will be excluded from the analysis.

Table 6. Systemic Event Grading Scale

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Grade 4
Fever	100.4-101.1°F (38.0-38.4°C)	101.2-102.0°F (38.5-38.9°C)	102.1-104.0°F (39.0-40.0°C)	> 104.0°F (> 40.0°C)
Vomiting	1-2 times in 24 hours	> 2 times in 24 hours	Requires IV[a] hydration	Emergency room visit or hospitalization for hypotensive shock
Diarrhea	2-3 loose stools in 24 hours	4-5 loose stools in 24 hours	≥ 6 loose stools in 24 hours	Emergency room visit or hospitalization
Headache	No interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency room visit or hospitalization
Fatigue/ Tiredness	No interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency room visit or hospitalization
New or worsening muscle pain	No interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency room visit or hospitalization
New or worsening joint pain	No interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency room visit or hospitalization

a. IV = intravenous.

For the data summary of the presence ("Yes" or "No") of a systemic event, the following variables for originally planned stage [first 3 vaccinations] and extension stage [fourth vaccination] are required:

- Presence ("Yes" or "No") of each systemic event on each day (Day 1 to Day 7 after the first vaccination on the Day 1, 8, and 30 regimen, Day 1 to Day 14 after all other vaccinations in both regimens).

- Presence ("Yes" or "No") of each systemic event on any day (Day 1 to Day 7 after the first vaccination on the Day 1, 8, and 30 regimen, Day 1 to Day 14 after all other vaccinations in both regimens).

- Presence ("Yes" or "No") of any systemic events on any day (Day 1 to Day 7 after the first vaccination on the Day 1, 8 and 30 regimen, Day 1 to Day 14 after all other vaccinations in both regimens).

- Presence ("Yes" or "No") of each systemic event with severe or greater severity (Grade 3 or Grade 4, as defined in Table 6) on each day (Day 1 to Day 7 after the first vaccination

on the Day 1, 8 and 30 regimen, and Day 1 to Day 14 after all other vaccinations in both regimens).

- Maximum severity of each systemic event on any day (Day 1 to Day 7 after the first vaccination on the Day 1, 8 and 30 regimen, and Day 1 to Day 14 after all other vaccinations in both regimens).

- Duration of each systemic event.

- Onset day of each systemic event since the last vaccination.

The algorithm for deriving the presence of a systemic event ("Yes" or "No") during Day 1 to Day 7 after the first vaccination on the Day 1, 8, and 30 regimen or during Day 1 to Day 14 after all other vaccinations in both regimens is similar to that in Table 3 and Table 4 for local reactions. For the purposes of deriving the variable "any systemic event on any day" (Day 1 to Day 7 after the first vaccination on the Day 1, 8, and 30 regimen or Day 1 to Day 14 after all other vaccinations in both regimens), for each day, a systemic event including fever, vomiting, diarrhea, headache, fatigue, new or worsening muscle pain, or new or worsening joint pain will be included as one of the possible systemic events.

In addition, similarly to local reactions, each systemic event, any systemic event, and maximum severity of each systemic event on any day after **any** vaccination in the original planned phase will be derived.

As stated before, only temperature data between 35.0°C and 42.0°C will be used for derivation of fever ("Yes" or "No") in "any systemic event on any day." The variable of "any systemic event on any day" will be derived similarly as "any local reaction on any day" based on the rule stated in Table 3. The duration of each systemic event and the onset day of each systemic event since the last vaccination are derived similarly as the rules stated above for local reactions. Antipyretics/pain medication used to treat symptoms will be handled separately and will not be considered as systemic events.

3.5.4. Antipyretics/Pain Medication Use

The following variables will be derived for the use of antipyretics/pain medication to treat symptoms:

- Medication use to treat fever or pain on each day (Day 1 to Day 7 after the first vaccination on the Day 1, 8, and 30 regimen, Day 1 to Day 14 after all other vaccinations in both regimens).

- Medication use to treat fever or pain on any day (Day 1 to Day 7 after the first vaccination on the Day 1, 8, and 30 regimen, Day 1 to Day 14 after all other vaccinations in both regimens).

- Duration of medication use to treat fever or pain.

- Onset day of medication use (since the last vaccination) to treat fever or pain.

Duration of medication use to treat fever or pain and onset day of medication use (since the last vaccination) to treat fever or pain are derived similarly as the rules stated above for local reactions.

3.5.5. Vital Signs and Physical Examination

Vital signs will be measured at Visit 1 and include weight, height, sitting blood pressure, sitting pulse rate, respiratory rate, and oral temperature. Body mass index (BMI) will be calculated based upon weight and height. Sitting blood pressure, sitting pulse rate, respiratory rate, and oral temperature (vaccination visits only) may be measured at each subsequent visit (Visit 2 to Visit 11 if clinically indicated. A full physical examination will be performed at Visit 1 and a targeted physical examination may be performed at each postrandomization visit (Visit 2 to Visit 11). All physical examination results will be recorded as normal, abnormal, or not done. Baseline measurements will be the measurements taken at Visit 1.

3.6. Study Conduct

3.6.1. E-Diary Completion

An e-diary will be considered transmitted if any data for the 3 local reactions, the 7 systemic events (including fever, vomiting, diarrhea, headache, fatigue, new or worsening muscle pain, and new or worsening joint pain), or use of antipyretics/pain medication to treat symptoms are present on any day. If all data are missing for all items on the e-diary for all days following vaccination (all 7 days following the first vaccination on the Day 1, 8, and 30 regimen, all 14 days following the first vaccination on the Month 0, 1, and 6 regimen, all 14 days following the second vaccination for both regimens, and all 14 days following the third vaccination for both regimens), then the e-diary will be considered not transmitted.

For transmitted e-diaries, the following variables will be defined: “Day 1,” “Day 2,” “Day 3,” “Day 4,” “Day 5,” “Day 6,” “Day 7,” “Day 8,” “Day 9,” “Day 10,” “Day 11,” “Day 12,” “Day 13,” “Day 14” (“Day 8” to “Day 14” only applicable to after Dose 2, Dose 3 on the Day 1, 8, and 30 regimen, and after each dose on the Month 0, 1, and 6 regimen), and Dose 4 in the extension stage in both regimens, “Day 1 – Day 7” (for after the first vaccination on the Day 1, 8, and 30 regimen), and “Day 1 – Day 14” (for after the second and the third vaccinations on the Day 1, 8, and 30 regimen, and after each vaccination on the Month 0, 1, and 6 regimen, and fourth vaccination in the extension stage of both regimens).

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 any given day, an e-diary will be considered complete if all expected data are available.

For completed e-diaries, the following variables will be defined for: “Day 1,” “Day 2,” “Day 3,” “Day 4,” “Day 5,” “Day 6,” “Day 7,” “Day 8,” “Day 9,” “Day 10,” “Day 11,” “Day 12,” “Day 13,” “Day 14” (“Day 8” to “Day 14” only applicable to after Dose 2, Dose 3 and Dose 4 in the extension stage of both regimens), “Day 1 – Day 7” (for after the first vaccination on the Day 1, 8, and 30 regimen), and “Day 1 – Day 14” (for after the second and the third vaccinations on the Day 1, 8, and 30 regimen, and after each vaccination on the Month 0, 1, and 6 regimen, and fourth vaccination in the extension stage of both regimens).

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

For after the first vaccination on the Day 1, 8, and 30 regimen:

= 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 or 1 day (<25%)

For after the second and after the third vaccinations on the Day 1, 8, and 30 regimen, and after each vaccination on the Month 0, 1, and 6 regimen, and fourth vaccination in the extension stage in both regimens:

= 1, if data have been transmitted, and are complete for 14 days (100%)

= 2, if data have been transmitted, and are complete for 11, 12, or 13 days (≥75% to <100%)

= 3, if data have been transmitted, and are complete for 7, 8, 9, or 10 days (≥50% to <75%)

= 4, if data have been transmitted, and are complete for 4, 5, or 6 days (≥25% to <50%)

= 5, if data have been transmitted, and are complete for 0, 1, 2, or 3 days (<25%)

3.6.2. Demographic, Medical History, and Baseline Characteristic Variables

The demographic variables are age at randomization/first vaccination visit (in years) at Visit 1, sex, race, ethnicity, and baseline serostatus of toxin A+/toxin B+, toxin A–/toxin B+, toxin A+/toxin B– , or toxin A–/toxin B–. Age will be calculated as (randomization/first vaccination date – date of birth + 1)/365.25 and truncated to the nearest lower integer. Medical history will be categorized according to MedDRA.

3.6.3. Concomitant Treatment

Nonstudy vaccine received from 6 months prior to enrollment until 1 month after the third vaccination (Visit 6 [Month 2] for subjects on the Day 1, 8, and 30 regimen and Visit 7 [Month 7] for subjects on the Month 0, 1, 6 regimen), and from 28 days prior to vaccination with the fourth vaccination until Visit 11(Day 30 after the fourth vaccination) for subjects in the extension stage of both regimens will be recorded on the CRFs.

Concomitant medications taken after signing the ICD until 1 month after the third vaccination (Visit 6 [Month 2] for subjects on the Day 1, 8, and 30 regimen and Visit 7 [Month 7] for subjects on the Month 0, 1, and 6 regimen) and from the time of the fourth vaccination until Visit 11(Day 30 after the fourth vaccination) for subjects in the extension stage of both regimens will be recorded on the CRFs. Nonstudy vaccines and concomitant medications will be categorized according to the World Health Organization (WHO) Drug Dictionary (WHODD).

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 (SOPs).

4.1. Evaluable Analysis Set

For each vaccination regimen, the evaluable immunogenicity population will be defined for each stage (original planned stage [first 3 vaccinations] and extension stage [fourth vaccination] separately.

For the Day 1, 8, and 30, regimen, the evaluable immunogenicity population in the original planned stage will include all subjects who:

1. Are eligible for the study at randomization.

2. Have received all 3 study vaccinations for the vaccine formulations to which they are randomized.

3. Have blood drawn for assay testing on Day 37 (Visit 5) within 7 to 14 days after the vaccination on Day 30 (Vaccination 3 at Visit 4) and the sample from this blood draw provides at least 1 valid and determinate assay result.

4. Have no major protocol violations as determined by the study clinician.

The evaluable immunogenicity population in the extension stage will include all subjects who:

1. Are eligible for the study extension stage at re-randomization.

2. Have received the fourth study vaccination with the vaccine formulations to which they are randomized.

3. Have blood drawn for assay testing on Day 8 after vaccination 4 (Visit 10) within 7 to 14 days after vaccination 4 (Visit 9) and the sample from this blood draw provides at least 1 valid and determinate assay result.

4. Have no major protocol violations as determined by the study clinician.

For the Month 0, 1, and 6 regimen, the evaluable immunogenicity population in the originally planned stage will include all subjects who:

1. Are eligible for the study at randomization.

2. Have received all 3 study vaccinations for the vaccine formulations to which they are randomized.

3. Have blood drawn for assay testing at Month 7 (Visit 7) within 20 to 45 days after the vaccination at Month 6 (Vaccination 3 at Visit 5) and the sample from this blood draw provides at least 1 valid and determinate assay result.

4. Have no major protocol violations as determined by the study clinician.

The evaluable immunogenicity population in extension stage will include all subjects who:

1. Are eligible for the study extension stage at re-randomization.

2. Have received fourth study vaccinations with the vaccine formulations to which they are randomized.

3. Have blood drawn for assay testing on Day 8 after vaccination 4 (Visit 10) within 7 to 14 days after vaccination 4 (Visit 9) and the sample from this blood draw provides at least 1 valid and determinate assay result.

4. Have no major protocol violations as determined by the study clinician.

The evaluable immunogenicity population will be the primary analysis population for all immunogenicity endpoints. The immunogenicity results will be summarized according to the vaccine group as randomly assigned.

Subjects vaccinated but not randomized and subjects randomized but who receive the wrong vaccine will be excluded from the evaluable immunogenicity population.

4.2. Modified Intent-to-Treat Analysis Set

For each vaccination regimen, the modified intent-to-treat (mITT) population will be defined for each stage separately. The mITT population for each regimen at each stage (original

planned stage or extension stage) will include all subjects randomized in that stage who have at least 1 valid and determinate assay result for the proposed analysis. This analysis set is for the purpose of immunogenicity analysis, for which it will be the secondary analysis population. In the immunogenicity data analysis based on the mITT population, results will be summarized according to the vaccine group as randomly assigned.

Subjects randomized but not vaccinated will be included in the mITT population for immunogenicity analysis if any valid and determinate assay results are available.

Subjects vaccinated but not randomized will be excluded from the mITT population.

4.3. Safety Analysis Set

The safety population will include all subjects who receive at least 1 dose of the investigational product. Separate safety populations will be defined for each regimen at each stage (original planned stage or extension stage). In the safety analysis based on the safety population, subjects will be grouped according to the vaccine actually received. Subjects who lack any safety data for a particular vaccination will be excluded from analysis.

Subjects vaccinated but not randomized will be included in the safety population for safety analysis, and results from these subjects will be reported under the group corresponding to the vaccine they actually received.

Subjects who were randomized but received the wrong vaccine will be included in the safety population for safety analysis, and results from these subjects will be reported under the group corresponding to the vaccine they actually received.

4.4. Other Analysis Sets

No other analysis sets will be defined.

5. GENERAL METHODOLOGY AND CONVENTIONS

The analysis for first clinical study report (CSR) will be performed when all subjects have completed Visit 9 and all immunogenicity and safety data up to and including Visit 9 are available. No multiplicity adjustments will be made on any of the descriptive analyses for safety or immunogenicity data. All CIs will be created at the nominal level in this analysis.

The analysis for the supplemental CSR will be performed when all extension-stage immunogenicity and safety data up to Visit 17 are available for subjects who entered the extension stage. Safety and immunogenicity data will be descriptively summarized periodically in order to protect the safety of subjects and to support internal program-level decision making. No multiplicity adjustments will be made on the descriptive analyses. All CIs will be created at nominal level in this analysis. For the originally planned stage, data will be analyzed separately for each 3-dose regimen: Days 1, 8, and 30 and Months 0, 1, and 6. For the extension stage, data will also be analyzed separately for originally assigned 3-dose regimen (Days 1, 8, and 30, Months 0, 1, and 6).

5.1. Hypotheses and Decision Rules

This is a Phase 2 study to assess the safety, tolerability, and immunogenicity of 2 antigen dose levels (100 µg and 200 µg total toxoid) of aluminum hydroxide–containing *C difficile* vaccine when administered as a 3-dose regimen: either at Days 1, 8, and 30 or at Months 0, 1, and 6. Subjects in both dosing regimens who received the first 3 doses of *C difficile* vaccine (100 µg or 200 µg) will be enrolled into an extension stage. These subjects will receive a fourth dose of either *C difficile* vaccine at the same antigen dose level (100 µg or 200 µg) as they received previously or placebo, approximately 1 year after their third dose. These subjects will be followed for a further 3 years (visits approximately every 6 months) to assess antibody persistence. Subjects originally randomized to placebo in either dosing regimen will not continue into the extension stage.

No formal statistical hypothesis test will be performed. A descriptive estimation approach will be used to assess all study objectives regarding safety and immunogenicity (PI, PS, SI, SS, and E) in the study.

Point estimates and nominal 95% CIs will be provided for all safety and immunogenicity endpoints at each planned analysis.

No formal multiplicity adjustments will be applied due to multiple endpoints or multiple analyses of the same endpoint.

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 the percentage (%) and the numerator (n) and the denominator (N) used in the percentage calculation.

5.2.1. Analyses for Binary Data

For immunogenicity results, the exact 2-sided 95% CIs (Clopper-Pearson CIs) will be provided for each vaccine group and sampling time point for the proportions of subjects, as defined in Section 3.2 with toxin A– and toxin B–specific neutralizing antibody levels greater than or equal to the specified thresholds, and with specified fold rise changes from baseline in toxin A– and toxin B–specific neutralizing antibody levels.

For each proportion of the immunogenicity results, a line plot of the proportions and the associated 95% CIs over the sampling time points will be provided by vaccine group (including placebo).

Similarly, for the safety results including research-related injury during the extension stage, the exact 2-sided 95% CIs will be provided by vaccine group for all primary safety endpoints, proportions of subjects reporting local reactions, systemic events, and AEs (including AEs occurring within the first 30 minutes after each vaccination and for the fourth dose in the extension stage).

For tier 1 AEs, an unconditional exact method for deriving the 95% CI for the risk difference and p-value proposed by Chan and Zhang (1999) will be used to compare vaccine with placebo.

For tier 2 AEs, the Miettinen and Nurminen method will be used to derive the 95% CI for the risk difference between vaccine and placebo.

The exact CIs for the various proportions of individual groups will be computed using the F distribution. If r is the number of responses and n is the number of subjects, then it follows that p=r/n is the estimate of the proportion of responses. An exact 95% CI can be computed by solving the following 2 equations. For the lower limit P_L,

$$p_L = \frac{rF_L}{(rF_L + (n - r + 1))}$$

and for the upper limit P_U,

$$p_U = \frac{(r+1)F_U}{(n-r) + (r+1)F_U}$$

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 should be set equal to 1.0 so P_L equals 0. When r equals n, F_U should be set equal to 1.0 so P_U equals 1.0.

The CI using the F distribution is described in Collett (1991) and implemented in SAS PROC FREQ.

5.2.2. Analyses for Continuous Data

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 for each vaccine group. The GMC will be calculated as the mean of the assay results after making the logarithm transformation and then back transformation to its original scale. Two-sided 95% CIs will be constructed by back transformation of the CI for the mean of the logarithmically transformed assay results computed based on the Student t distribution.

For each *C difficile* toxin A– and toxin B–specific neutralizing antibody levels, a line plot of the GMC and the associated 95% CI over the sampling time points will be provided by vaccine group, separately for each assigned 3-dose regimen in the original planned stage and for the fourth dose in the extension stage.

The GMFRs in toxin A– and toxin B–specific neutralizing antibody levels from before vaccination to after vaccination will be summarized for each vaccine group by geometric means and associated CIs at all postbaseline blood sampling time points. These CIs are also computed by back transformation of the CIs using the Student t distribution for the mean difference of measures on the logarithmically transformed assay results.

For the originally planned stage, empirical reverse cumulative distribution curves (RCDCs) will be presented graphically for each *C difficile* toxin A– and toxin B–specific neutralizing antibody levels for each vaccine group (including placebo) at Day 37 (Visit 5) and all blood sampling time points after Day 37 for the Day 1, 8, and 30 regimen, and at Month 7 (Visit 7) and all blood sampling time points after Month 7 for the Month 0, 1, and 6 regimen.

For the extension stage, RCDCs will also be presented graphically for each *C difficile* toxin A– and toxin B–specific neutralizing antibody level for each vaccine group at Day 8 after Vaccination 4 (Visit 10) and all blood sampling time points after Visit 10.

5.3. Methods to Manage Missing Data

5.3.1. Immunogenicity Data

For any *C difficile* toxin A– or toxin B–specific neutralizing antibody level that is below the LLOQ, the lower limit of detection (LOD), defined as 0.5 × LLOQ, will be assigned. No other missing assay data will be imputed in the analyses. All immunogenicity analyses will be performed after the imputation of the antibody levels that are below the LLOQ.

For each *C difficile* toxin A– or toxin B–specific neutralizing antibody result, the number of subjects with missing values at each blood sampling point will be provided.

5.3.2. Safety Data

Handling of missing information related to safety data, such as missing or partially missing date, will be in accordance with Pfizer reporting standards.

For derived variables in reactogenicity data, if any day of the 7-day e-diary is available, the "Day 1 – Day 7" data will be considered as nonmissing for analyses after Vaccination 1 on the Day 1, 8, and 30 regimen and if any of the 14-day e-diary is available, the "Day 1 – Day 14" data will be considered as nonmissing for analyses after Vaccinations 2 and 3 on the Day 1, 8, and 30 regimen, and after each vaccination on the Month 0, 1, and 6 regimen, and Vaccination 4 in the extension stage. The proportion of subjects with missing reactogenicity data will also be summarized by each vaccination for each vaccine group. The denominator will be the number of subjects who receive the scheduled vaccination.

6. ANALYSES AND SUMMARIES

6.1. Primary Endpoint(s)

6.1.1. Primary Immunogenicity Endpoints

6.1.1.1. Primary Analysis

Endpoints: Primary immunogenicity endpoints in Section 3.1.1

- Analysis time points:
  For the Day 1, 8, and 30 regimen: Day 37
  For the Month 0, 1, and 6 regimen: Month 7
- Analysis populations: Evaluable immunogenicity and mITT populations
- Analysis methodology: See Section 5.2.1
- Supporting objective: Primary immunogenicity objectives

Reporting Results:

The numerator and denominator used for the proportion and associated 95% CI at Day 37 or Month 7 will be presented for each vaccine group in each regimen (Days 1, 8, and 30 or Months 0, 1, and 6).

Figures:

The antibody response line plot of proportions and the associated 95% CI at Day 37 or Month 7 will be provided by vaccine group, separately for each regimen.

6.1.2. Primary Safety Endpoints

Endpoints: Primary safety endpoints in Section 3.1.2

- Analysis time points**:**
  Local reactions and systemic events: Up to 14 days following each vaccination
  AEs: From the first vaccination up to 28 days after the third vaccination
  SAEs: From the first vaccination until 6 months after the third vaccination
- Analysis population**:** Safety population
- Analysis methodology**:** Descriptive; see Section 5.2.1
- Supporting objective: Primary safety objective

Reporting Results:

The number, proportion, and associated 95% CI will be presented for each vaccine group in each regimen (Days 1, 8, and 30 or Months 0, 1, and 6).

6.2. Secondary Endpoints

6.2.1. Secondary Immunogenicity Endpoints

Endpoints: Secondary immunogenicity endpoints described in Section 3.2

Endpoints: GMCs for toxin A– and toxin B–specific neutralizing antibody levels

- Analysis time points**:**

  Original planned stage:

  For the Day 1, 8, and 30 regimen: Days 1, 8, 15, 30, and 37 and Months 2, 4, 7, and 13
  For the Month 0, 1, and 6 regimen: Days 1, 30, and 37, Months 2 and 6, Day 187, and Months 7, 12, and 18

  Extension stage:
  at the fourth dose (immediately before the fourth dose), at Days 8 and 30 after the fourth dose, and at Months 6, 12, 18, 24, 30, and 36 after the fourth dose:

- Analysis populations**:** mITT and evaluable immunogenicity populations

- Analysis methodology**:** See Section 5.2.2

- Supporting objective: Secondary immunogenicity objective

Reporting Results:

The number, GMC, and associated 95% CI at the above analysis time points will be presented for each vaccine group in each regimen (Days 1, 8, and 30 or Months 0, 1, and 6) separately for the originally planned stage and the extension stage

Figures:

The antibody-response line plot of GMCs and the associated 95% CI for each toxin A– and toxin B–specific neutralizing antibody level at the above analysis time points will be presented by vaccine group, separately for each regimen in the originally planned stage and the extension stage.

Endpoints: GMFRs from baseline in toxin A– and toxin B–specific neutralizing antibody levels

- Analysis time points**:**

  Original planned stage:
  For the Day 1, 8, and 30 regimen: Days 8, 15, 30, and 37, and Months 2, 4, 7 and 13
  For the Month 0, 1, and 6 regimen: Days 30 and 37, Months 2 and 6, Day 187, and Months 7, 12, and 18

  Extension stage:
  at the fourth dose (immediately before the fourth dose), at Days 8 and 30 after the fourth dose, and at Months 6, 12, 18, 24, 30, and 36 after the fourth dose

- Analysis populations**:** mITT and evaluable immunogenicity populations

- Analysis methodology**:** See Section 5.2.2

- Supporting objective: Secondary immunogenicity objective

Reporting Results:

The sample size, GMFR, and associated 95% CI at the above analysis time points will be presented for each vaccine group in each 3-dose regimen (Days 1, 8, and 30 or Months 0, 1, and 6) separately for the originally planned stage and the extension stage.

Endpoints: Proportions with ≥4-fold, ≥8-fold, ≥16-fold, and ≥32 fold rises from baseline in toxin A–specific, toxin B–specific, and both toxin A– and toxin B–specific neutralizing antibody levels

- Analysis time points**:**

  Original planned stage:
  For the Day 1, 8, and 30 regimen: Days 8, 15, 30, and 37 and Months 2, 4, 7, and 13
  For the Month 0, 1, and 6 regimen: Days 30 and 37, Months 2 and 6, Day 187, and Months 7, 12, and 18

  Extension stage:
  at the fourth dose (immediately before the fourth dose), at Days 8 and 30 after the fourth dose, and at Months 6, 12, 18, 24, 30, and 36 after the fourth dose

- Analysis populations**:** mITT and evaluable immunogenicity populations

- Analysis methodology**:** See Section 5.2.1

- Supporting objective: Secondary immunogenicity objective

Reporting Results:

The numerator and denominator used for proportion, proportion, and associated 95% CI at the above analysis time points will be presented for each vaccine group in each regimen (Days 1, 8, and 30 or Months 0, 1, and 6) separately for the originally planned stage and the extension stage.

Endpoints: Proportions with toxin A–specific neutralizing antibody levels ≥ the specified threshold for toxin A; proportions with toxin B–specific neutralizing antibody levels ≥ the specified threshold for toxin B; proportions with both toxin A– and toxin B–specific neutralizing antibody levels ≥ the specified threshold for toxin A and the specified threshold for toxin B, respectively.

- Analysis time points**:**

  Original planned stage:
  For the Day 1, 8, and 30 regimen: Days 1, 8, 15, and 30 and Months 2, 4, 7, and 13
  For the Month 0, 1, and 6 regimen: Days 1, 30, and 37, Months 2 and 6, Day 187, and Months 12 and 18

  Extension stage:
  at the fourth (immediately before the fourth dose), at Days 8 and 30 after the fourth dose, and at Months 6, 12, 18, 24, 30, and 36 after the fourth dose

- Analysis populations**:** mITT and evaluable immunogenicity populations

- Analysis methodology**:** See Section 5.2.1

- Supporting objective: Secondary immunogenicity objective

Reporting Results:

The numerator and denominator used for proportion, the proportion, and associated 95% CI at the above analysis time points will be presented for each vaccine group in each regimen (Days 1, 8, and 30 or Months 0, 1, and 6) separately for the originally planned stage and the extension stage.

Figures:

The antibody-response line plot of proportions and the associated 95% CI at the above analysis time points will be presented by vaccine group, separately for each regimen in the originally planned stage and the extension stage.

6.2.2. Secondary Safety Endpoints

Endpoints: Secondary safety endpoints in Section 3.2.2

- Analysis time points**:**
  Local reactions and systemic events: Up to 14 days following fourth vaccination
  AEs: From the time of the fourth vaccination up to 28 days after the fourth vaccination
  SAEs: From the time of the fourth vaccination up to 28 days after the fourth vaccination

- Analysis population**:** Safety population

- Analysis methodology**:** Descriptive; see Section 5.2.1

- Supporting objective: Secondary safety objective

Reporting Results:

The number, proportion, and associated 95% CI will be presented for each vaccine group in each originally assigned regimen (Days 1, 8, and 30 or Months 0, 1, and 6).

6.3. CCI [redacted]

CCI [redacted]

[redacted]

[redacted]

Table 7. CCI [redacted]

CCI [redacted]

Table 7. CCI [redacted]

CCI [redacted]

Subgroup analyses of primary and selected secondary immunogenicity endpoints (proportions equal to or above the prespecified threshold, proportions achieving >= 4 fold from baseline and GMCs) will be conducted by age cohorts (65 to 69 years, 70 to 74 years, 75 to 79 years, and 80 to 85 years), separately for each regimen, to explore the impact of age on the immunogenicity results. Similar subgroup analyses (proportions equal to or above the prespecified threshold, proportions achieving >= 4 fold from baseline and GMCs) will be conducted by the combination of age cohorts and baseline status. All subgroup analyses will be performed based on the evaluable immunogenicity population.

For the statistical analysis related to endpoints of proportions of subjects achieving 1) toxin A–specific, 2) toxin B–specific, and 3) toxin A– and toxin B–specific neutralizing antibody levels ≥ the specified thresholds defined in Section 3.1.1, alternative levels/thresholds may be considered.

6.4. Baseline and Other Summaries and Analyses

6.4.1. Baseline Summaries

The number and percentage of subjects with baseline serostatus of toxin A+/toxin B+, toxin A–/toxin B–, toxin A+/toxin B–, and toxin A–/toxin B+ will be provided and included in a subject demographic summary table.

6.4.2. Study Conduct

6.4.2.1. Subject Disposition, Vaccination Administration, Blood Samples, and Screen Failures

The number and percentage of subjects who are randomized will be included in the subject disposition summary for the originally planned stage: subjects who withdraw during the vaccination phase (Visit 1 to Visit 4 on the Day 1, 8 and 30 regimen; Visit 1 to Visit 5 on the Month 0, 1, and 6 regimen), complete the vaccination phase, , and withdraw after the vaccination phase will be summarized. The number and percentage of subjects who are re randomized will be included in the subject disposition summary for the extension stage: the number and percentage of subjects who complete the study and withdraw after fourth vaccination will be summarized. 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.

Subjects excluded from the evaluable immunogenicity population will also be summarized with reasons for exclusion.

The number and percentage of subjects randomized, vaccinated, and providing blood samples within the protocol-specified time frame, as well as before and after the specified time frame, will be tabulated for the originally planned stage. In addition, the number and percentage of subjects rerandomized, vaccinated, and providing blood samples within the protocol-specified time frame, as well as before and after the specified time frame, will be tabulated for the extension stage.

A listing of noncompliant vaccine administration will be provided. The protocol deviations will also be listed. Subjects who do not receive the vaccine as randomized will be listed as well. A listing of subjects who withdrew because of AEs will be provided.

All randomized subjects will be used to generate these tables. All of the summary tables will be presented for each vaccine group and for the total, separately for each assigned 3-dose regimen in the originally planned stage and the extension stage.

Subjects who sign the ICD but are screen failures will be also summarized separately for each assigned 3-dose regimen in the originally planned stage.

6.4.2.2. Demographic, Medical History, and Baseline Characteristics

Descriptive summary reports for demographic characteristics will be provided for each vaccine group, separately for each regimen in the originally planned stage for the evaluable immunogenicity and mITT analysis populations.

Descriptive summary reports for medical history will be provided for each vaccine group, separately for each regimen for the safety population only in the originally planned stage.

6.4.2.3. E-Diary Completion

Variables defined in Section 3.6.1 will be summarized with descriptive statistics for each vaccine group and for the total after each dose, separately for each regimen in the originally planned stage and the extension stage. The safety population will be used to generate this table.

6.4.3. Analyses of Below LLOQ

A table summarizing the number of subjects with assay data below the LLOQ will be produced for each vaccine group separately for each regimen in the originally planned stage and the extension stage. This table will be generated for both the evaluable and mITT immunogenicity populations.

6.5. Safety Summaries and Analyses

All safety analyses will be summarized based on the safety population in accordance with Pfizer reporting standards. All safety summaries will be provided by vaccine group separately for each dose in each 3-dose regimen (Days 1, 8, and 30 and Months 0, 1, and 6) in the originally planned stage and for each original assigned regimen in the extension stage.

6.5.1. Adverse Events

It should be recognized that most studies are not designed to reliably demonstrate a causal relationship between the use of a pharmaceutical product and an AE or a group of AEs. Except for select events in unique situations, studies do not employ formal adjudication procedures for the purpose of event classification. As such, safety analysis is generally considered as an exploratory analysis and its purpose is to generate hypotheses for further investigation. The 3-tier approach facilitates this exploratory analysis. There will be no adjustment for multiple comparisons in the analyses.

For tier 1 and tier 2 events, the proportion of AEs observed in each vaccine group will be presented along with the point estimates and associated 95% CIs of the risk difference (the difference of incidence rates) between each vaccine and placebo.

For tier 1 events, 95% CIs will be calculated using the exact methods proposed by Chan and Zhang described in Section 5.2.1.

For tier 2 events, 95% CIs will be calculated using the Miettinen and Nurminen method described in Section 5.2.1.

For tier 1 events, p-values from Chan and Zhang's method will be calculated and included in the presentations. AEs will be arranged in the output sorted in descending order of point estimates of the risk difference (the difference of incidence rates) within system organ class.

6.5.2. Reactogenicity Data

The derived endpoints (Section 3.5.3) for each local reaction, systemic event, and use of antipyretics/pain medication to treat symptoms will be summarized.

The presence and maximum severity of the local reaction and systemic event at "any day (Day 1 – Day 7)" following the first vaccination on the Day 1, 8, and 30 regimen "any day (Day 1 – Day 14)" following the second and third vaccinations on the Day 1, 8, and 30 regimen, and "any day (Day 1 – Day 14)" following each vaccination on the Month 0, 1 and 6 regimen in originally planned stage and fourth vaccination in the extension stage for both regimens will be summarized with 95% Clopper-Pearson CIs.

The presence of each local reaction and systemic event on any day (Day 1- X), any local reaction on any day (1-X), maximum severity of each local reaction and systemic event on any day (Day 1-X) following **any** vaccination on the Day 1, 8, and 30 regimen and the Month 0, 1, and 6 regimen in the originally planned stage will also be summarized with Clopper-Pearson CIs, where X = 7 days after the first vaccination on the Day 1, 8 and 30 regimen, and X = 14 after all other vaccinations in both regimens. For local reactions, systemic events, and use of antipyretic/pain medication to treat symptoms, descriptive summary statistics of the maximum duration of the event will be provided.

For the onset of local reactions, systemic events, and use of antipyretic/pain medication to treat symptoms, descriptive summary statistics of the onset day will be provided.

6.5.2.1. Unscheduled Visits (Unplanned Visits) for Severe Reactions

A listing will be generated for all of the subjects with unscheduled/unplanned visits because of severe (Grade 3) and Grade 4 reactions.

6.5.3. Immediate AEs

Descriptive summaries and listing of subjects reporting AEs during the protocol-specified first 30-minute observation period will be summarized by vaccine group after each dose for the assigned 3-dose regimen in the originally planned stage and also summarized by vaccine group for each original assigned 3-dose regimen in the extension stage . Also, Clopper-Pearson 95% CIs will be included with the percentages.

CCI [redacted]

6.5.5. Physical Examinations, Including Vital Signs

Descriptive summary tables will be provided in accordance with Pfizer reporting standards.

6.5.6. Nonstudy Vaccination and Nonstudy Medication

Nonstudy vaccination and nonstudy medication summaries will be provided with descriptive summaries.

7. INTERIM ANALYSES

7.1. Introduction

This is a Phase 2, placebo-controlled, randomized, observer-blinded study.

In addition to the planned analyses described in Section 7, unblinded safety data from the study will be reviewed by an external data monitoring committee (DMC) approximately twice a year as part of program-level safety review activities, governed by a program-level DMC charter.

For each analysis described in this SAP, the analysis data sets used to create the tables, listings, and figures will be provided to the appropriate sponsor personnel to support the planned data review and decision making.

7.2. Interim Analyses and Summaries

Four interim analyses are planned for the study and described as below. An internal review committee (IRC) will be established to review the interim analyses results and make recommendations. The IRC may request and review additional analysis as needed. An IRC charter will be finalized prior to the first interim analysis with details of the interim analyses objectives, decision guidance, and method of maintaining the study blinded following appropriate Pfizer's SOPs.

A list of protocol deviations will be compiled prior to the first planned interim analysis, and will be updated prior to each follow-up interim analysis and the final analysis. Subjects who do not meet the eligibility criteria and subjects who have major protocol violations will be excluded from the evaluable immunogenicity population. A major protocol violation is a protocol violation that, in the opinion of the clinicians, would materially affect assessment of immunogenicity, eg, subject receipt of a prohibited vaccine or medication that might affect immune response or a medication error with suspected decrease in potency of the vaccine. Clinicians will identify those subjects with protocol violations before each of the interim analyses and the final analysis of immunogenicity results.

7.2.1. First Interim Analysis

The first analysis is planned for the study when Month 2 data are available from all continuing subjects of both regimens. All available immunogenicity and safety data, for both vaccination regimens, will be included in this analysis.

The objective of the first analysis is to determine the suitability of the Day 1, 8 and 30 regimen for further study.

7.2.2. Second Interim Analysis

The second interim analysis is planned for the study when Month 7 data are available from approximately the first 700 continuing subjects. All available immunogenicity and safety data will be included in this analysis.

The objective of the second analysis is to inform internal program-level decision making.

7.2.3. Third Interim Analysis

The third interim analysis is planned for the study when Month 7 data are available from all continuing subjects of both regimens. All available immunogenicity and safety data, for both vaccination regimens, will be included in this analysis.

The objective of the third analysis is to determine the suitability of the Month 0, 1 and 6 regimen for further study and to select the optimal vaccine dose level for further study.

7.2.4. Fourth Interim Analysis

The fourth interim analysis will be conducted when 6 months post–Dose 3 data are available from all continuing subjects (Month 7 for subjects receiving the Day 1, 8 and 30 regimen and Month 12 for subjects receiving the Month 0, 1 and 6 regimen). All available immunogenicity and safety data will be include in the primary analysis.

The objective of the fourth analysis is to support regulatory interactions.

8. REFERENCES

1. Collett D. Modelling binary data. London: Chapman & Hall; 1991.

2. Chan ISF, Zhang Z. Test-based exact confidence intervals for the difference of two binomial proportions. Biometrics 1999;55:1201-9.

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