# Efficacy, Immunogenicity, and Safety Study of *Clostridium difficile* Vaccine in Subjects at Risk for *C. difficile* Infection (Cdiffense™)

Randomized, observer-blind, placebo-controlled, multi-center, multi-national, Phase III trial in 16,500 subjects

# Statistical Analysis Plan (SAP) - Core Body Part

| | |
|---|---|
| **Trial Code:** | H-030-014 |
| **Development Phase:** | Phase III |
| **Sponsor:** | Sanofi Pasteur |
| **Investigational Product:** | *Clostridium difficile* Toxoid Vaccine |
| **Author:** | [redacted] |
| **Form/Route:** | Intramuscular (IM) |
| **Indication For This Study:** | Prevention of primary *C. difficile* infection (CDI) |
| **Version and Date of the SAP core body part:** | Version 4.0 date 5 September 2017 |

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# List of Abbreviations

| | |
|---|---|
| AE | adverse event |
| BL | blood sample |
| CI | confidence interval |
| CRF | case report form |
| CRO | Contract Research Organization |
| D | day |
| DC | diary card |
| eCRF | electronic case report form |
| EDC | electronic data capture |
| ELISA | enzyme linked immunosorbent assay |
| FAS | full analysis set |
| GMC | geometric mean concentration |
| GMT | geometric mean titer |
| ICH | International Conference on Harmonization |
| IDMC | Independent Data Monitoring Committee |
| IgG | Immunoglobulin G |
| IVRS | interactive voice response system |
| IWRS | interactive web response system |
| LLOQ | lower limit of quantitation |
| MAR | Missing at Random |
| MD | missing data |
| MedDRA | Medical Dictionary for Regulatory Activities |
| MITT | modified intent-to-treat |
| PCR | polymerase chain reaction |
| PP | per-protocol analysis set |
| Q1; Q2; Q3 | first quartile; second quartile (median); third quartile |
| RCDC | reverse cumulative distribution curve |
| SAE | serious adverse event |
| SafAS | safety analysis set |
| SAP | statistical analysis plan |
| SPUS | Sanofi Pasteur unblinded statistician |
| TNA | toxin neutralization assay |
| ULOQ | upper limit of quantitation |
| VE | vaccine efficacy |

# 1 Introduction

Sanofi Pasteur is developing a toxoid vaccine against primary symptomatic *Clostridium difficile* infection (CDI). The vaccine target indication is for the prevention of CDI primary occurrence in at-risk individuals. At-risk individuals are defined as adults that require frequent and or prolonged antibiotic use and exposure to the healthcare environment, which may include hospitalization and/or long-term care, nursing home, or rehabilitation admission.

*C. difficile* is the most common cause of infectious nosocomial diarrhea and is responsible for up to 30% of all diarrhea cases among hospitalized patients (1). The primary reservoirs of *C. difficile* within hospitals and long-term care facilities include colonized or infected patients and contaminated environments and surfaces (1) (2). Once colonized, patients with *C. difficile* shed spores in their feces. The incidence is also increasing among persons living in the community with recent healthcare contact (3). All pathogenic strains of *C. difficile* produce toxin B with or without toxin A, causing a variety of syndromes which vary widely in clinical spectrum and severity (diarrhea, colitis, pseudomembranous colitis, toxic megacolon, and death) (4).

There is a lack of standardized reporting mechanisms and surveillance definitions of CDI in most countries worldwide. The worldwide CDI incidence in the general hospital population has been estimated to range between 5 to 10 per 10,000 patients-days (5) (6) (7). In 2008, in a prospective incidence survey including 34 European countries, the overall incidence of hospital-acquired CDI was estimated at 4.1 cases per 10,000 patient-days and ranged from 0 to 36.3 among the different countries (7).

The incidence and the severity of CDI have been increasing in recent years and remain underestimated (8). CDI attributed mortality is difficult to estimate due to the high level of co-morbidities among CDI patients. However, the estimated overall mortality attributable to CDI is approximately 6%, rising to 13% to 15% in older patients (9).

Until recently, therapeutic options for the treatment of CDI have been limited to a few antibacterials, such as vancomycin, metronidazole and bacitracin. Fidaxomicin was approved in 2011 for the treatment of CDI in adults. For patients with non-severe disease, these treatments all provide effective treatment. Several other CDI targeted antimicrobial and non-antimicrobial agents are in various stages of development as current therapeutics are suboptimal secondary to the high incidence of relapsing disease and the potential for emergence of drug-resistance in non-clostridial bacteria.

There is strong evidence that these host immune responses to *C. difficile* toxins A and B have a substantial role in determining the clinical outcome of *C. difficile* infection. Serum anti-toxin immunoglobulin G (IgG) antibody levels have been found to play an important role in determining the outcome of colonization and protection against a first episode of CDI. Patients who are asymptomatically colonized with *C. difficile* have higher serum anti-toxin A IgG levels, measured by enzyme-linked immunosorbent assay (ELISA), than colonized patients who develop diarrhea (10). This suggests that high anti-toxin A IgG levels at the time of colonization protect against CDI. The effectiveness of serum anti-toxin antibodies in protection from symptoms in colonized individuals and serum anti-toxin A and B antibodies in reduction of recurrent disease support the hypothesis that systemic humoral immunity against *C. difficile* toxins will prevent the manifestations of CDI in humans. It is anticipated that through active vaccination, the *C. difficile* Toxoid Vaccine will elicit broader anti-toxin responses and immunological memory.

# 2 Trial Objectives

## 2.1 Primary Objective

To assess the efficacy of the *C. difficile* vaccine in preventing the onset of symptomatic primary CDI confirmed by polymerase chain reaction (PCR) in adult subjects aged ≥ 50 years who are at risk for CDI and have received at least 1 injection.

## 2.2 Secondary Objectives

Efficacy:

- To assess prevention of symptomatic PCR-confirmed primary CDI cases after 3 injections administered at 0, 7, and 30 days
- To assess prevention of symptomatic PCR-confirmed primary CDI cases after completion of at least 2 injections
- To assess durability of prevention of symptomatic PCR-confirmed primary CDI cases up to 3.0 years after the third injection
- To assess prevention of severe primary CDI cases in subjects with PCR-confirmed primary CDI
- To assess the effect of the vaccine on reduction of loose stool frequency in subjects who are symptomatic primary PCR-confirmed CDI cases
- To assess the effect of the vaccine on reduction of CDI episode/illness duration in subjects who are symptomatic primary PCR-confirmed CDI cases

Immunogenicity:

- To describe the immunogenicity to toxin A and toxin B:
  - in the subset (1,650 out of16,500) of subjects and in subjects with CDI (250) at Day(D) 0 and D60 (± 14 days)

    and
  - in the subset (1,650 out of 16,500) of subjects at D14 (+3 days), D30 (–3 days to +7 days), and every 6 months up to 3.0 years (± 14 days) after the third injection.

Safety:

- To describe the safety profile of all subjects who receive at least 1 injection.

## 2.3 Exploratory Objectives

[redacted]

# 3 Description of the Overall Trial Design and Plan

## 3.1 Trial Design

This is a randomized, observer-blind, placebo-controlled, multi-center, multi-national Phase III trial in 16,500 subjects. Adult subjects aged ≥ 50 years who are at risk for CDI will be enrolled.

Subjects will be enrolled in 1 of 2 risk strata across the treatment groups.

Risk Stratum 1:

- Has had at least 2 hospital stays, each lasting at least ≥ 24 hours, in the 12 months before enrollment

and

- Has received systemic (not topical) antibiotics in the 12 months before enrollment

or

Risk Stratum 2:

- Is anticipated to have an in-patient hospitalization for a planned surgical procedure within 60 days of enrollment and the impending hospital stay is planned to be ≥ 72 hours (see also Inclusion Criteria in the protocol).

For stratification purposes, subjects who meet the criteria for both Risk Strata will be assigned to Risk Stratum 1.

The study is designed as an event-driven, group sequential protocol with, 4 interim analyses at 50, 100, 135, 167 clinical endpoints (i.e., PCR-confirmed CDI episodes) and a final analysis. Subjects will be randomly assigned in a 2:1 ratio to receive either vaccine or placebo. Vaccine or placebo

will be administered in a 3-dose schedule on Days 0, 7, and 30. At the time of group assignment, 1650 subjects (10% of total enrollment) will be randomly assigned to an immunogenicity subset. 3,300 subjects (20% of total enrollment) will be randomly assigned to a reactogenicity subset , All subjects will provide blood samples for immunogenicity assessment at D0 (pre-injection) and D60. The immunogenicity subset from both study groups will also provide blood samples at D14, at D30 pre-injection, and every 6 months through the planned follow-up periods (i.e., up to 3.0 years after the third injection).

In a reactogenicity subset solicited AEs will be collected for 6 days following each injection. AEs will be collected for all subjects from D0 to D60. All SAEs will be collected through the end of the follow-up period.

All subjects will be actively followed for efficacy with contact every 2 weeks throughout the study and follow-up period. This follow-up period may extend for up to 3.0 years after the last injection.

Analyses of trial futility (non-efficacy) will be performed at the first 2 interim analyses. The study may be stopped if either of those analyses provides robust and compelling evidence that meaningful levels of vaccine efficacy (VE) will not be demonstrated. The 95% confidence interval (CI) of the observed VE will be calculated at the 1st (50 cases) and 2nd (100 cases) interim analyses. Futility will be claimed if the upper bound of the 95% VE CI is less than 46%.

Analyses of efficacy will be performed at the 2nd, 3rd and 4th interim analyses as well as the final analysis with the total one-side type I error rate at 0.025 . The nominal significance levels for these 3 efficacy interim analyses are 0.01, 0.0035 and 0.0032, respectively. The nominal significance levels are determined by the corresponding proportion of information assessed at each interim analysis (number of new cases at each interim / 250 * 0.025). The remaining alpha (0.0083) after the interim analyses will be used at the final analysis. Early termination for declaration of efficacy at any interim analysis will be made if the lower bound of VE CI at its associated nominal significance level is greater than 15%. .

In addition to the aforementioned statistical criteria, a minimum of 12 months median follow-up duration after the last injection will be required to stop the trial early for efficacy.

**Note*: There are imperfections in the sensitivity and specificity of PCR. Given this, an observed 48%, 46%, and 42% VE correspond to an estimated true VE of 63%, 60%, and 55%.

## 3.2 Trial Plan

Eligible subjects will be identified and enrolled. Each subject must sign and date the informed consent form before any procedure or treatment associated with the study is performed.

Subjects will receive one injection of either *C. difficile* Toxoid Vaccine or placebo on Days 0, 7, and 30.

For subjects in the main study, blood (20 mL/bleed) will be collected before injection at Visit 1 (D0) and at Visit 4 (60 days [+14] days after injection).

At enrollment, 10% of subjects will be randomly assigned by interactive voice response system (IVRS)/ interactive web response system (IWRS) to an immunogenicity subset. For these subjects, blood (20 mL/bleed) for serologic testing will be collected before injection at Visit 1

(D0) and at Visit 3 (D14), Visit 4 (D30), Visit 5 (D60), Visit 6 (D210), and then every 6 months as long as the subject is in the trial.

At enrollment, 20% of subjects will be randomly assigned by IVRS/IWRS to a reactogenicity subset of 3,300 subjects. These subjects will be asked to record solicited reactions for 6 days after each injection.

All subjects will record unsolicited AEs that occur from D0 through D60 (30 days after the last injection). All subjects will be followed for safety for at least 6 months after the last injection.

Subjects will be contacted on D210 (±14 days) and up to 3.0 years (±14 days) to ascertain whether any additional SAEs not already reported occurred since the last contact. All SAEs (related and unrelated) will be collected in all subjects from D0 through the end of the follow-up period. During the follow-up period, subjects will be instructed to contact the clinical site if they are hospitalized or admitted to a long-term care or rehabilitation facility, or if they experience an AE that might be considered serious.

All subjects will be followed for the follow-up period (up to 3.0 years after the third injection) for occurrence of repeated episodes of loose stools and provision of a stool sample. For all subjects, there will be a weekly contact (telephone, home or clinic visit) for 4 weeks after enrollment (D0 through D30) or following discharge from a hospital or long-term care or rehabilitation facility, to remind subjects to report repeated episodes of loose stools and provide stool samples, if applicable. Following the first 4 weeks, subjects will be contacted every 2 weeks for the duration of the study and follow-up period The method of contact may be by telephone, text message, email, and/or home visit; however, there must be subject contact via telephone or home visit once a month.

If a subject is hospitalized, or is admitted to a long-term care or rehabilitation facility, the subject will be contacted once a week until discharge or for a maximum of 3 months to monitor the subjects for episodes of loose stools, for which a stool sample will be obtained and provided to the site. After discharge from the hospital or facility, the subjects will be contacted weekly for 4 weeks, then every 2 weeks thereafter for the remainder of the follow-up period as described above. Details of the surveillance plans are provided in the Operating Guidelines that will be given to the investigational sites before the start of the study.

Additionally, a quality of life questionnaire will be completed following a repeated episode of loose stools for which a sample is collected. When possible, subjects will complete the survey on their own or via telephone interview with the site staff. Two other quality of life questionnaires will be completed within 5 to 6 and 8 to 9 weeks after the beginning of each episode. For subjects in the immunogenicity subset, a quality of life questionnaire will also be completed at Visits 3, 6, 8, and 10.

The questionnaire may be completed by the subject or may be administered and recorded by site staff via telephone or may be completed by a proxy in case the subject's condition is such that he or she is not able to answer.

# Table of Study Procedures for the Main Population:

(N=14,850 out of a total of 16,500)*

Phase III Trial, minimum of 4 Visits, 3 Injections, a minimum of 2 Blood Samples, Telephone Calls/Contacts, 3.0 years Duration Per Subject

**Note:* There is a separate Table of Study Procedures for subjects in the immunogenicity subset (N=1,650)

| **Visit** | **Visit 1** | **Visit 2** | **Visit 3** | **Visit 4** | **Contact** | **Contact** |
|---|---|---|---|---|---|---|
| **Trial timelines (days or years)** | Day 0 | Day 7 | Day 30 | Day 60 | Day 210 | up to 3.0 years after scheduled Visit 3 (D30) |
| **Time windows (days)** | - | +3 days | −3 to +7 days | +14 days | ±14 days | ±14 days |
| Informed consent | X | | | | | |
| Inclusion/exclusion criteria | X | | | | | |
| Collection of demographic data | X | | | | | |
| Urine pregnancy test[*] | X | X | X | | | |
| Medical history | X | | | | | |
| Contraindications | | X | X | | | |
| Physical examination[†] | X | X | X | | | |
| Temperature | X | X | X | | | |
| Randomization/allocation of subject number and unique product kit number | X | | | | | |
| Allocation of a unique product kit number | | X | X | | | |
| Blood sample (BL), 20 mL/bleed | BL1[‡] | | | BL2 | | |
| **Injection (vaccine or placebo)** | X | X | X | | | |
| Immediate surveillance (30 min) | X | X | X | | | |
| Diary card (DC) or memory aid (MA) provided[§] | DC1 | DC2 | DC3 | MA | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| Memory aid for loose stools (MA-LS)§ | MA-LS | | | | | |
| Diary card (DC) collected§ | | DC1 | DC2 | DC3 | | |
| Collection of solicited injection site & systemic reactions** | 6 days after each injection | | | | | |
| Collection of unsolicited AEs§ | From Day 0 through 30 days after the last injection | | | | | |
| Collection of reportable concomitant medications | X | X | X | X | X†† | X†† |
| Telephone call | | | | | X | |
| Trial termination record‡‡ | | | | | | X |
| Provision of stool sample§§ | Throughout the trial | | | | | |
| Surveillance*** | Throughout the trial | | | | | |
| Completion of quality of life questionnaire††† | Throughout the trial | | | | | |
| Collection of SAEs‡‡‡ | Throughout the trial | | | | | |

* For women of child-bearing potential. Urine sample obtained and tested pre-injection.

† All physical examinations will be performed pre-injection. A body system targeted physical examination will be performed prior to each injection based on the subject's medical history and the examiner's medical judgment.

‡ Blood sample collected pre-injection.

§ Diary cards and memory aid:

**DC1:** For all subjects, collection of unsolicited AEs and concomitant medications, and, for subjects in the reactogenicity subset, collection of solicited reactions for 6 days after the first-injection. Completed DC1 will be reviewed and collected by the site at Visit 2.

**DC2:** Collection of unsolicited AEs and concomitant medication for Visit 2 through Visit 3 (around Day 7 through Day 30), and, for subjects in the reactogenicity subset, collection of solicited reactions for 6 days after the second injection. DC2 will be reviewed and collected by the site at Visit 3.

**DC3:** Collection of unsolicited AEs and concomitant medication from Visit 3 through Visit 4 (around 30 days after the last injection), and, for subjects in the reactogenicity subset, collection of solicited reactions for 6 days after the third injection, The subject will be reminded by telephone during routine surveillance call (around Day 43) to bring the DC to the site at Visit 4 (Day 60).

**MA:** Collection of medical events and/or hospitalization and medications at the time of the event for 6 months after the third injection. The MA will be reviewed at the Day 210 safety follow-up telephone call. Caller will ascertain whether any SAEs occurred since the last contact. During the follow-up period, subjects will be instructed to contact the clinical site if they are hospitalized or admitted to a long-term care or rehabilitation facility or experience an AE that might be considered serious.

**MA-Loose Stools:** Maintained by subject throughout trial duration to record date and time of each loose stool episode and temperature during the episode If a loose stool episode is reported by subject to clinical staff, clinical staff will review dates and times of each episode and record in source documents and the eCRF(s).

** Solicited reactions will be recorded only by those subjects who were randomly assigned to the reactogenicity subset.

†† Only Category 2 and 3 concomitant medications will be collected after Day 60.

‡‡ The trial termination record should be completed at the time of final contact or at the time of early termination.

§§ Will be collected from subjects who experience repeated episodes of loose stools.

*** All subjects will be contacted weekly (by telephone, home or clinic visit) for 4 weeks after enrollment (Day 0 through Day 30) and for the 4 weeks immediately following discharge from documented periods of hospitalization or long-term care or rehabilitation facility, to remind subjects to report episodes of loose stools. Following this 4-week period, all subjects will be contacted every 2 weeks. The method of contact can vary (telephone, text message, email, and/or home visit); however, there must be subject contact via telephone or home visit once a month.

During a hospitalization or admission to long-term care or rehabilitation facility, the subject will be contacted weekly until discharge or for a maximum of 3 months for any repeated episodes of loose stools for which a stool sample will be obtained and provided to the site.

All subjects will be actively followed with contact every 2 weeks (as described above) for the follow-up period for occurrence of repeated episodes of loose stools.

††† Completed 3 times if a repeated episode of loose stools occurs: once at the time of each episode, once 5 to 6 weeks after the beginning of each episode, and once 8 to 9 weeks after the beginning of each episode.

‡‡‡ There will be a safety follow-up phone call approximately 6 months after the third injection. Study staff will ascertain whether any SAEs occurred since the last contact.

During the follow-up period, subjects will be instructed to contact the clinical site if they are hospitalized or admission to long-term care or rehabilitation facility or experience an AE that might be considered serious.

# Table of Study Procedures for the Immunogenicity Subset:

(N=1,650 out of a total of 16,500)

Phase III Trial, 10 or 11 Visits during injection phase, 3 Injections, a minimum of 10 Blood Samples, Telephone Calls/Contacts, 3.0 years Duration Per Subject

| **Visit** | **Visit 1** | **Visit 2** | **Visit 3** | **Visit 4** | **Visit 5** | **Visit 6** | **Visit 7** | **Visit 8** | **Visit 9** | **Visit 10** | **Visit 11** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Trial timelines (days)** | Day 0 | Day 7 | Day 14 | Day 30 | Day 60 | Day 210 | Day 390 (1.0 yr after Visit 4) | Day 570 (1.5 yr after Visit 4) | Day 750 (2.0 yr after Visit 4) | Day 930 (2.5 yr after Visit 4) | Day 1110 (3.0 yr after Visit 4) |
| **Time windows (days)** | - | +3 days | +3 days | −3 to +7 days | +14 days | ±14 days | ±14 days | ±14 days | ±14 days | ±14 days | ±14 days |
| Informed consent | X | | | | | | | | | | |
| Inclusion/exclusion criteria | X | | | | | | | | | | |
| Collection of demographic data | X | | | | | | | | | | |
| Urine pregnancy test* | X | X | | X | | | | | | | |
| Medical history | X | | | | | | | | | | |
| Contraindications | | X | | X | | | | | | | |
| Physical examination† | X | X | | X | | | | | | | |
| Temperature | X | X | | X | | | | | | | |
| Randomization/allocation of subject number and unique product kit number | X | | | | | | | | | | |
| Allocation of unique product kit number | | X | | X | | | | | | | |
| Blood sample (BL), 20 mL/bleed | BL1‡ | | BL2 | BL3‡ | BL4 | BL5 | BL6 | BL7 | BL8 | BL9 | BL10 |
| **Injection (vaccine or placebo)** | X | X | | X | | | | | | | |
| Immediate surveillance (30 min) | X | X | | X | | | | | | | |
| Diary card (DC) or memory aid (MA) provided§ | DC1 | DC2 | | DC3 | MA | | | | | | |
| Diary card (DC) reviewed | | | DC2 | | | | | | | | |
| Diary card (DC) collected§ | | DC1 | | DC2 | DC3 | | | | | | |
| Memory aid for loose stools (MA-LS)§ | MA-LS | | | | | | | | | | |

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Collection of unsolicited AEs[§] | From Day 0 through 30 days after the last injection | | | | | | | | | | |
| Collection of reportable concomitant medications | X | X | X | X | X | X[**] | X[**] | X[**] | X[**] | X[**] | X[**] |
| Trial termination record[††] | | | | | | | | | | | X |
| Provision of stool sample[‡‡] | Throughout the trial | | | | | | | | | | |
| Surveillance[§§] | Throughout the trial | | | | | | | | | | |
| Completion of quality of life questionnaire[***] | Throughout the trial | | | | | | | | | | |
| Collection of SAEs[†††] | Throughout the trial | | | | | | | | | | |

[*] For women of child-bearing potential. Urine sample obtained and tested pre-injection.

[†] All physical examinations will be performed pre-injection. A body system targeted physical examination will be performed prior to each injection based on the subject's medical history and the examiner's medical judgment.

[‡] Blood sample collected pre-injection.

[§] Diary cards and memory aid:

**DC1:** Collection of unsolicited AEs and concomitant medication, and, for subjects in the reactogenicity subset, collection of solicited reactions for 6 days after the first-injection. Completed DC1 is reviewed and collected by the site at Visit 2 (Day 7).

**DC2:** Collection of unsolicited AEs and concomitant medication for Visit 2 through Visit 4, and, for subjects in the reactogenicity subset, collection of solicited reactions for 6 days after the second injection. DC2 will be reviewed at Visit 3 (Day 14), returned to the subject, and then reviewed and collected by the site at Visit 4 (Day 30).

**DC3:** Collection unsolicited AEs and concomitant medication for Visit 4 through Visit 5, and, for subjects in the reactogenicity subset, collection of solicited reactions for 6 days after the third injection. DC3 will be reviewed and collected by the site at Visit 5 (Day 60).

**MA:** Collection of medical events and/or hospitalization and medications at the time of the event for 6 months after the third injection (Day 60 through Day 210). The MA will be reviewed at the Day 210 safety follow-up visit. Caller will ascertain whether any SAEs occurred since the last contact. During the follow-up period, subjects will be instructed to contact the clinical site if they are hospitalized or experience an AE that might be considered serious.

**MA-Loose Stools:** Maintained by subject throughout trial duration to record date and time of loose stool episode and temperature during the episode. If a loose stool episode is reported by subject to clinical staff, clinical staff will review dates and times of each episode and record in source documents and the eCRF(s).

[**] Only Category 2 and 3 concomitant medications will be collected after Day 60.

[††] The trial termination record should be completed at the time of final contact or at the time of early termination.

[‡‡] Will be collected from subjects who experience repeated episodes of loose stools

[§§] All subjects will be contacted weekly (by telephone, home, or clinic visit) for 4 weeks after enrollment (Day 0 through Day 30) and for the 4 weeks immediately following discharge from documented periods of hospitalization or admission to a long-term care facility or rehabilitation facility, to remind subjects to report episodes of diarrhea. Following this 4-week period, all subjects will be contacted every 2 weeks. The method of contact can vary (telephone, text message, email, and/or home visit); however, there must be subject contact via telephone or home visit once a month.

During a hospitalization or admission to long-term care facility or rehabilitation facility, the subject will be contacted weekly until discharge or for a maximum of 3 months for any repeated

episodes of loose stools for which a stool sample will be obtained and provided to the site.
For subjects in the immunogenicity subset, there will be a home or clinic visit every 6 months during the follow-up period after the last injection for collection of a blood sample.

[***] Completed 4 times: at Visit 3, Visit 6, Visit 8, and Visit 10. Also completed 3 times if a repeated episode of loose stools occurs: once at the time of each episode, once 5 to 6 weeks after the beginning of each episode, once 8 to 9 weeks after the beginning of each episode.

[†††] There will be a safety follow-up phone call approximately 6 months after the third injection. Study staff will ascertain whether any SAEs occurred since the last contact. During the follow-up period, subjects will be instructed to contact the clinical site if they are hospitalized or admission to long-term care or rehabilitation facility or experience an AE that might be considered serious.

# 4 Endpoints and Assessment Methods

## 4.1 Primary Endpoints and Assessment Methods

### 4.1.1 Efficacy Endpoints

The primary endpoints for the evaluation of efficacy (i.e., symptomatic PCR-confirmed primary CDI cases) are:

- Presence of both of the following clinical symptoms:
  - ≥ 3 loose stools in ≤ 24-hours
  - loose stools lasting ≥ 24 hours

  *Notes:*
  - Timing of 24-hour period will start from the first episode of loose stools
  - Loose stool is defined as type 6 (fluffy pieces with ragged edges, a mushy stool) or type 7 (watery, no solid pieces; entirely liquid) according to the Bristol Stool Chart (11).

and

- Stool sample positive for *C. difficile* by PCR

or

Confirmatory test of pseudomembranous colitis diagnosed through colonoscopy, and, if available, provision of a stool sample for PCR-testing

### 4.1.2 Efficacy Assessment Methods

PCR will be performed according to the laboratory protocol at the Contract Research Organization (CRO) testing site.

## 4.2 Secondary Endpoints and Assessment Methods

### 4.2.1 Efficacy Endpoints

- The number of symptomatic PCR-confirmed primary CDI cases after 3 injections (the per-protocol [PP] population)
- The number of symptomatic PCR-confirmed primary CDI cases after at least 2 injections
- The number of symptomatic PCR-confirmed primary CDI cases after 3 injections as a function of time since enrollment and within 3.0 years after the third injection
- The number of severe PCR-confirmed primary CDI cases. A severe case is defined when a subject has 1 or more of the following: fever ≥ 38.5°C , white blood cell count ≥ 15,000 cells/$mm^3$ (if available), ileus, pseudomembranous colitis, serum albumin <3 g/dl, abdominal distension, abdominal tenderness, or admission to the intensive care unit within 7 days of CDI diagnosis
- The maximum number of loose stools per day associated with a symptomatic PCR-confirmed primary CDI case

- The CDI episode/illness duration associated with a symptomatic PCR-confirmed primary CDI case. Duration is calculated as (clinical cure date - clinical case date + 1)

### 4.2.2 Efficacy Assessment Methods

PCR will be performed according to the laboratory protocol at the CRO testing site.

### 4.2.3 Immunogenicity Endpoints

- Serum antibody concentrations against toxins A and B, measured by ELISA
- Serum antibody titers against toxins A and B, measured by TNA
- [redacted]

### 4.2.4 Immunogenicity Assessment Methods

***ELISA testing:***

Serum will be tested by ELISA for IgG antibodies to *C. difficile* toxin A and toxin B to generate primary immunogenicity data. Additional evaluations of antibody responses may be performed to further characterize the immune response to vaccination with *C. difficile* Toxoid Vaccine, which may include other serum immunoglobulins to *C. difficile* toxin A and toxin B.

The principle of the ELISA for the detection of human IgG antibodies to *C. difficile* toxin A or B antigens involves the reaction of antibodies present in the test sera with the toxin A or B antigens adsorbed to the individual wells of a microtiter test plate. The amount of antibody bound to the toxin A antigen coated wells is determined by a colorimetric substrate reaction after the binding of a secondary anti-human IgG antibody-enzyme conjugate. Substrate for the enzyme is added which causes colorimetric change that is directly proportional to the antibody bound to the antigen. The concentration of antibodies in serum is then derived by extrapolation from a standard curve, which is generated from multiple dilutions of a reference standard serum with a defined IgG unitage (ELISA units [EU]/ml).

Each specimen will be processed according to directions provided in the Operating Guidelines. Appropriate training for specimen processing will be administered prior to study initiation.

***Toxin neutralization assay:***

This is a cell-based cytotoxicity neutralization assay. The assay can quantitate neutralizing antibodies to *C. difficile* toxin by incubating serial diluted serum with *C. difficile* toxin A or B. Vero cells are then added and this serum-toxin-cell mixture is incubated at 37°C for 6 days. The ability of the sera to neutralize the cytotoxic effect of the *C. difficile* toxin is determined by and correlated to the viability of the Vero cells.

The test utilizes the accumulation of acid metabolites in closed culture wells as an indication of normal cell respiration. In cells exposed to toxin, metabolism and $CO_2$ production is reduced; consequently, the pH rises to 7.4 or higher as indicated by the phenol red pH indicator in the cell culture medium. At this pH, the medium appears red. Control cells, or cells exposed to toxin which has been neutralized by antibody, however, metabolize and produce $CO_2$ in normal amounts; as a result, the pH is maintained at 7.0 or below. At this pH, the medium appears yellow. Therefore, *C. difficile* toxin neutralizing antibodies correlate with the ability of the serum to

neutralize the metabolic effects of *C. difficile* toxin on cells as evidenced by their ability to maintain a pH of 7.0 or lower. The color change of the media can be measured at 562-630 nm by a plate reader to further calculate the antitoxin neutralizing antibody titer at 50% inhibition of the *C. difficile* toxin-mediated cytotoxicity.

Each specimen will be processed according to directions provided in the Operating Guidelines. Appropriate training will be administered prior to study initiation.

### 4.2.5 Safety Endpoints

The secondary endpoints for the evaluation of safety are:

- Occurrence, nature (Medical Dictionary for Regulatory Activities [MedDRA] preferred term), duration, intensity, and relationship to vaccination of any unsolicited systemic AEs reported in the 30 minutes after vaccination
- Occurrence, time to onset, number of days of occurrence, intensity, action taken, and whether the reaction led to early termination from the study, of solicited (prelisted in the subject's diary card and eCRF) injection site reactions occurring up to 6 days after vaccination for subjects in the reactogenicity subset
- Occurrence, time to onset, number of days of occurrence, intensity, action taken, and whether the reaction led to early termination from the study, of solicited (prelisted in the subject's diary card and eCRF) systemic reactions occurring up to 6 days after vaccination for subjects in the reactogenicity subset
- Occurrence, nature (MedDRA preferred term), time to onset, duration, intensity, action taken, relationship to vaccination (for systemic AEs only), and whether the event led to early termination from the study, of unsolicited AEs up to 30 days after vaccination
- Occurrence, nature (MedDRA preferred term), time to onset, duration, seriousness criteria, relationship to vaccination, outcome, and whether the SAE led to early termination from the study, of SAEs through the end of the trial after the last injection
- Occurrence, nature (MedDRA preferred term), time to onset, duration, seriousness criteria, outcome, and whether the SAE led to early termination from the study, of all SAEs through the end of the follow-up period

### 4.2.6 Safety Assessment Methods

#### 4.2.6.1 Immediate Post-vaccination Surveillance Period

Subjects will be kept under observation for 30 minutes after each vaccination to ensure their safety. Any AE that occurs during this period will be noted on the source document and identified as an immediate event / reaction; and will additionally be recorded in the eCRF, as follows:

- Any unsolicited systemic AE observed to occur during the first 30 minutes post-vaccination will also be recorded on the eCRF, i.e., as immediate unsolicited systemic AE.
- Solicited and unsolicited injection site reactions and solicited systemic reactions will not be actively solicited during the 30 minute-period. If they occur within 30 minutes of vaccination, they will be recorded and analyzed as starting on the day of vaccination.

- Any SAE occurred during the first 30 minutes post-vaccination will be reported in the same way as any other SAE and to the Sponsor

#### 4.2.6.2 Reactogenicity (Solicited Reactions from Day 0 to Day 6 after Each Vaccination)

For those subjects (20% of the total enrollment) randomly assigned to the reactogenicity subset of 3,300 subjects, after the first visit, subjects will be provided with a safety diary card, a digital thermometer, and a flexible ruler, and will be instructed how to use them. The following items will be recorded by the subjects in the diary card on the day of vaccination and for the next 6 days (i.e., D0 to D6) until resolution:

- Daily temperature, with the route by which it was taken
- Daily measurement or intensity grade of all other solicited injection site and systemic reactions
- Action taken for each event, if any (e.g., medication)

Table 4.1 and Table 4.2 present, respectively, the injection site reactions and systemic reactions that are prelisted in the diary cards and CRF, together with the intensity scales.

The doses on Days 0 and 7 will be injected into alternate arms, if possible (i.e., the vaccination nurse will use the opposite arm each time). If the vaccine is administered in alternate arms, then the injection site reactions after each dose can be independently assessed. If it is not possible to administer vaccine in alternate arms, then the same arm may be used for consecutive vaccinations. The D30 dose may be injected into either arm.

For solicited systemic reactions and unsolicited systemic AEs, if a reaction/event is ongoing at the time of the next dose, it will be treated differently depending on whether or not it has increased in intensity following the later dose. If it has not increased in intensity, it is to be attributed to the earlier dose, and is recorded as just a single AE. If it has increased in intensity, it will be treated as 2 AEs: the date of the later dose will be considered to be the end date for the first AE and the start date of the second AE.

**Table 4.1: Solicited injection site reactions: terminology, definitions, and intensity scales**

| MedDRA term | Injection site pain | Injection site erythema | Injection site swelling |
|---|---|---|---|
| **Diary card term** | Pain | Redness | Swelling |
| **Definition** | | Presence of a redness including the approximate point of needle entry | Swelling at or near the injection site<br>Swelling or edema is caused by a fluid infiltration in tissue or cavity and, depending on the space available for the fluid to disperse, swelling may be either soft (typically) or firm (less typical) to touch and thus can be best described by looking at the size of the swelling |
| **Intensity scale** * | Grade 1: No interference with activity<br>Grade 2: Some interference with activity<br>Grade 3: Significant; prevents daily activity | Grade 1: ≥ 25 to ≤ 50 mm<br>Grade 2: > 51 to ≤ 100 mm<br>Grade 3: > 100 mm | Grade 1: ≥ 25 to ≤ 50 mm<br>Grade 2: > 51 to ≤ 100 mm<br>Grade 3: > 100 mm |

* For the subjective reaction of pain, subjects will record the intensity level (Grade 1, 2, or 3) in the diary card. For the measurable reactions of redness and swelling, they will record just the size of the reaction, and the classification as Grade 1, 2, or 3 will be assigned by the statistician.

## Table 4.2: Solicited systemic reactions: terminology, definitions, and intensity scales

| **CRF term (MedDRA lowest level term [LLT])** | **Fever** | **Headache** | **Malaise** | **Myalgia** | **Arthralgia** |
|---|---|---|---|---|---|
| **Diary card term** | Temperature | Headache | Feeling unwell | Muscle aches and pains | Joint pain |
| **Definition** | Elevation of temperature to ≥°38.0° (≥ 100.4°F) | Pain or discomfort in the head or scalp. Does not include migraine. | General ill feeling.<br>Malaise is a generalized feeling of discomfort, illness, or lack of well-being that can be associated with a disease state. It can be accompanied by a sensation of exhaustion or inadequate energy to accomplish usual activities. | Muscle aches and pains are common and can involve more than one muscle at the same time. Muscle pain can also involve the soft tissues that surround muscles. These structures, which are often referred to as connective tissues, include ligaments, tendons, and fascia (thick bands of tendons).<br>Does not apply to muscle pain at the injection site which should be reported as injection site pain. | Pain in more than 2 major joints, including shoulders, elbows, wrists, hips, knees, and ankles |
| **Intensity scale *** | Grade 1: ≥38.0°C to ≤ 38.4°C **or** ≥ 100.4°F to ≤ 101.1°F<br>Grade 2: ≥ 38.5°C to ≤ 38.9°C **or** ≥101.2°F to ≤102.0°F<br>Grade 3: ≥39.0°C **or** ≥102.1°F | Grade 1: No interference with activity<br>Grade 2: Some interference with activity<br>Grade 3: Significant; prevents daily activity | Grade 1: No interference with activity<br>Grade 2: Some interference with activity<br>Grade 3: Significant; prevents daily activity | Grade 1: No interference with activity<br>Grade 2: Some interference with activity<br>Grade 3: Significant; prevents daily activity | Grade 1: Free range of motion but complains of pain or discomfort<br>Grade 2: Decreased range of motion due to pain or discomfort<br>Grade 3: Unwilling to move due to pain |

* For all reactions but fever, subjects will record the intensity level (Grade 1, 2, or 3) in the diary card. For fever, they will record the body temperature, and the classification as Grade 1, 2, or 3 will be assigned by the statistician.

***Important notes for the accurate assessment of temperature:***

Subjects are to measure body temperature once per day, preferably always at the same time. The optimal time for measurement is the evening, when body temperature is the highest. Temperature is also to be measured at the time of any apparent fever. The observed daily temperature and the route of measurement are to be recorded in the diary card, and the highest temperature will be recorded by the site in the eCRF. The preferred route for this trial is oral; however, temperature may be measured according to local practice. The route of temperature measurement will be recorded in the eCRF. Tympanic thermometers must not be used.

#### 4.2.6.3 Unsolicited Adverse Events from Day 0 to Day 30 after Each Vaccination

All subjects will be instructed to record any unsolicited AEs that may occur during the 30-day period after each vaccination. Space will be provided in the diary card for this purpose. For each AE, the following information is to be recorded:

- Start and stop dates[1]
- Intensity of the event
- For measurable unsolicited AEs that are part of the list of solicited reactions, the size of the AE as well as the temperature for fever will be collected and analyzed based on the corresponding scale used for solicited reactions (see Table 4.1 and Table 4.2).
- Other unsolicited non-serious AEs will be classified according to the following intensity scale:

  Grade 1: No interference with activity

  Grade 2: Some interference with activity

  Grade 3: Significant; prevents daily activity
- Action taken for each AE, if any (e.g., medication)
- Whether the AE led to discontinuation

AEs likely to be related to the product, whether serious or not, that persist at the end of the trial will be followed up by the Investigator until their complete disappearance or the stabilization of the subject's condition. The Investigator will inform the Sponsor of the date of final disappearance of the event.

#### 4.2.6.4 Serious Adverse Events

Information on all SAEs will be collected and assessed throughout the trial from inclusion through the end of the follow-up period.

Any SAE occurring at any time during the trial will be reported by the Investigator through the EDC system and according to the completion guidelines provided by the Sponsor. All information concerning the SAE is to be reported, either as part of the initial reporting or during follow-up

[1] The stop date of all related AEs will be actively solicited. For other events, the investigator will provide the stop date when it becomes available. AEs for which no stop date was obtained during the course of the trial will be considered as ongoing at the end of the trial.

reporting if relevant information became available later (e.g., outcome, medical history, results of investigations, copy of hospitalization reports). The Investigator will assess the causal relationship between the SAE and the investigational product as either "Not related" or "Related".

#### 4.2.6.5 Assessment of Causality

At each vaccination visit, the Investigator or a delegate will perform a clinical or medically-driven physical examination, and will ask the subject about any solicited reactions (only for those subjects in the reactogenicity subset) and unsolicited AEs recorded in the diary card, as well as about any other AEs that may have occurred since the previous visit. All relevant data will be transcribed into the eCRF according to the instructions provided by the Sponsor.

The action taken by the subject to treat any **solicited reactions** will be classified in the eCRF using the following scale:

0: None
1: Medication (self-medication with an existing prescription or over-the-counter medication)
2: Health care provider contact (no new medication prescribed)
3: Health care provider contact and prescription of a new medication (health care provider instructed subject to take a new medication, either an over-the-counter medication or one requiring a written prescription)
4: Hospitalization (inpatient)

The action taken by the subject to treat any **unsolicited AEs** will be classified in the eCRF using the following scale:

0: None
1: Medication (self-medication with an existing prescription or over-the-counter medication)
2: Health care provider contact (no new medication prescribed)
3: Health care provider contact and prescription of a new medication (health care provider instructed subject to take a new medication, either an over-the-counter medication or one requiring a written prescription)

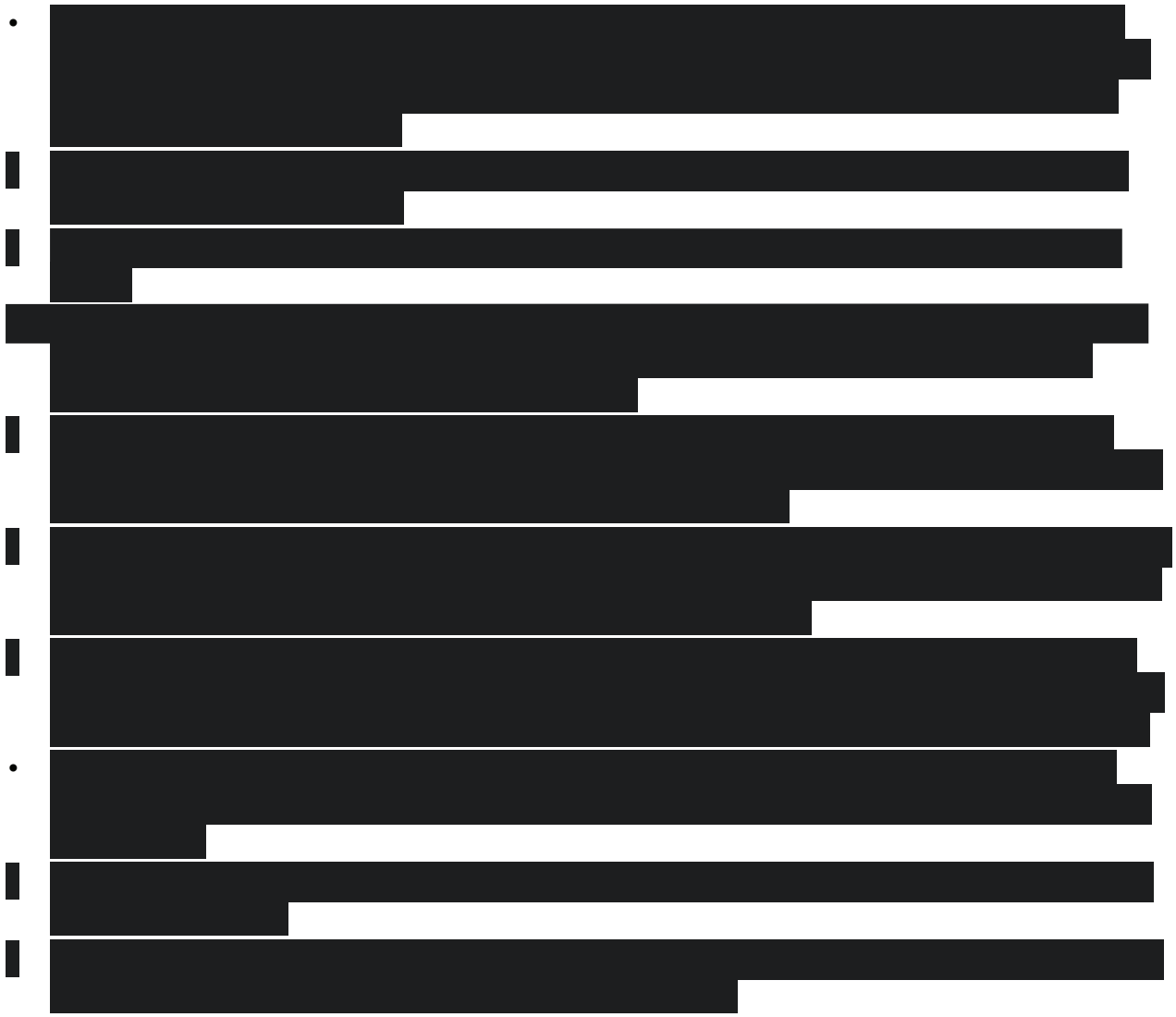
The Investigator will assess the ***causal relationship*** between each unsolicited systemic AE and vaccination as either not related or related, based on the following definitions[2]:

0: Not related – The AE is clearly / most probably caused by other etiologies such as subject's underlying condition, therapeutic intervention, or concomitant therapy; or the delay between vaccination and the onset of the AE is incompatible with a causal relationship; or the AE started before the first vaccination (screening phase, if applicable)
1: Related – There is a "reasonable possibility" that the AE was caused by the vaccination, meaning that there is evidence or arguments to suggest a causal relationship

[2] ICH Guidelines, Clinical Safety Data Management E2A

*Note:* By convention, all injection site AEs (solicited and unsolicited) and all solicited systemic reactions are considered to be related to vaccination and referred to as reactions, and therefore do not require the Investigator's opinion on relatedness.

## 4.3 Exploratory Endpoints and Assessment Methods



## 4.4 Derived Endpoints: Calculation Methods

### 4.4.1 Safety

#### 4.4.1.1 Solicited Reactions

For each solicited reaction, subjects are included in the analysis if they provided any data for the reaction. Thus the denominator for each solicited reaction is the number of subjects with either non-missing presence data, non-missing daily intensity data or non-missing ongoing data for the reaction.

The conventions for imputing a value of "Missing" in Section 4.4.1.1.1 to Section 4.4.1.1.6 below apply only to tables; in listings, data will be shown as reported.

For solicited systemic reactions (and injection site reaction if needed), following the 1st injection, if a reaction is ongoing at the time of the 2nd injection with no increase in intensity, the subject will be counted in only the 1st injection. If the intensity increases the subject will be counted in both injections, the date of the 2nd injection will be considered to be the end date for the first reaction and the start date of the second reaction.

For example,

**Table 4.3 Calculating intensity**

| | Injection 1 | | | | | | | Injection 2 | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **Day 0** | **Day 1** | **Day 2** | **Day 3** | **Day 4** | **Day 5** | **Day 6** | **Day 0** | **Day 1** | **Day 2** | **Day 3** | **Day 4** | **Day 5** | **Day 6** |
| **Intensity example 1** | 0 | 1 | 2 | 2 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 0 |
| **Intensity example 2** | 0 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | 2 | 1 | 1 | 0 | 0 | 1 |
| **Intensity example 3** | 0 | 1 | 2 | 2 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 |

- For intensity example 1, the subject will be counted in both injections (new reaction started on D1 following the 2nd injection; reaction durations of 5 and 4 days following the 1st and 2nd injection, respectively)
- For intensity example 2, the subject will be counted in both injections (intensity increases at D2 of the 2nd injection; reaction durations of 6 and 7 days following the 1st and 2nd injection, respectively)
- For intensity example 3, the subject will be counted in the 1st injection only (intensity did not increase following 2nd injection; reaction duration of 10 days following the 1st injection)

#### 4.4.1.1.1 Daily Intensity

The intensity will be recorded by the investigator for AEs whose scale is not a measure. For measurable AEs, the intensity will be calculated at the time of statistical analysis.

The maximum intensity during a period will be computed without considering the ongoing period.

A reaction that is too large to measure (reported as 'non measurable', NM) is assumed to be Grade 3.

Missing measurements for temperature or length will not be replaced. Nevertheless the following rule will be applied: If a temperature or length is partially missing (missing after the decimal point), the data will be analyzed replacing "MD" (MD means missing data) by zero (whatever the group). For example, a "39.MD" daily temperature will be considered as "39.0°C" at the time of analysis.

#### 4.4.1.1.2 Presence

Presence will be computed based on daily measurements during the solicited period. For any specific period, a subject would be considered to have a reaction if the intensity is greater than or equal to Grade 1 for at least one day during that period.

If no data is recorded for a specific period, the solicited reaction will be considered as missing for that period.

#### 4.4.1.1.3 Time to Onset

In time to onset tables, the time to onset will be the number of days from vaccination to the first occurrence Grade 1 or higher (i.e., first day with intensity different from none or missing)[3].

Time to onset will be displayed by periods as D0 to D3 and D4 to D7.

#### 4.4.1.1.4 Number of Days of Occurrence

In tables, the number of days of occurrence will be determined by the number of days with intensity different from none or missing between D0 and the end of the solicited period.

If the reaction is ongoing after the solicited period, the number of days of occurrence will be calculated as follows:

- Number of days of occurrence = (stop date • last vaccination date) + (number of days of occurrence between D0 and D7) • 8 + 1

If the reaction is ongoing (i.e. Maximum intensity after D7 at least Grade 1), and the stop date is unfilled (‘blank’) or missing (containing ‘MD’), then the number of days of occurrence after the solicited period will be considered as missing.

#### 4.4.1.1.5 Stop Dates

Stop date is the last date the reaction is present. Missing or incomplete stop dates after D7 will not be imputed.

#### 4.4.1.1.6 Action Taken

Action taken for each reaction will be reported (see Section 4.2.6.5). Missing action taken will not be imputed.

### 4.4.1.2 Unsolicited Non-serious AEs and SAEs

Subjects are included in the analysis of unsolicited events if they received the study injection; thus the denominator is the number of subjects who received the study injection.

For unsolicited systemic AEs, if an event is ongoing at the time of the next dose, it will be treated differently depending on whether or not it has increased in intensity following the later dose. If it

[3] Note: If an event is not continuous (i.e., an event occurs over two separate periods of time intervened by at least one day of missing or zero (0) intensity) then consider only the first day of the first occurrence (non-zero and non-missing intensity).

has not increased in intensity, it is to be attributed to the earlier dose, and is recorded as just a single AE. If it has increased in intensity, it will be treated as 2 AEs: the date of the later dose will be considered to be the end date for the first AE and the start date of the second AE.

Unsolicited adverse reactions are unsolicited AEs considered related to vaccination, either as determined by the investigator, or because they occurred at the injection site.

The conventions for imputing a value of "Missing" in Section 4.4.1.2.1 to Section 4.4.1.2.7 below apply only to tables; in listings, data will be shown as reported.

In general, unsolicited AEs will include immediate AEs and SAEs.

#### 4.4.1.2.1 Causality

AEs considered related to vaccination, either as determined by the investigator, or because they occurred at the injection site. Unsolicited non-serious AEs and SAEs with missing causality will be considered as related to vaccination.

#### 4.4.1.2.2 Intensity

In tables, the intensity will be recorded by the investigator for AE whose scale is not a measure. For measurable AE, the intensity will be calculated at the time of statistical analysis.

Missing measurements (for temperature or length) will not be replaced.

Note for SAEs: Intensity will not be collected.

#### 4.4.1.2.3 Start Date

Start date is the first date the event was present. Unsolicited events with a missing or partial start date will be assumed to have occurred between D0 and D30 and thus will be included in tables of events between these two days.

#### 4.4.1.2.4 Last Vaccination before Unsolicited AE or SAE

The last vaccination before AE or SAE is defined as the most recent vaccination received by the subject before the event is present.

#### 4.4.1.2.5 Action Taken

Action taken for each reaction will be reported, see Section 4.2.6.5. Missing action taken will not be imputed.

#### 4.4.1.2.6 Outcome (for SAEs)

Missing outcome will not be imputed for SAEs.

#### 4.4.1.2.7 Seriousness (for SAEs)

An SAE may have more than one seriousness category. Missing seriousness will not be imputed.

#### 4.4.1.2.8 Stop Date

Stop date is the first date the event was not present. Unsolicited events with a missing or partial stop date that were reported during the course of the trial will be considered as ongoing at the end of the trial.

### 4.4.2 Immunogenicity

Serum will be tested by ELISA for IgG antibodies and by cell-based cytotoxicity toxin neutralization assay (TNA) to *C. difficile* toxin A and toxin B to generate the primary immunogenicity data.

#### 4.4.2.1 Computed Values for Analysis

A $\log_2$ transformation will be performed on available data titers to calculate geometric mean titer (GMT) and geometric mean concentration (GMC).

At each time point, each criterion described in Section 5.1.2.5 will be derived by a binary variable, except for the GMT calculations.

For the imputation of values to assay results reported as < lower limit of quantitation (LLOQ), see Section 5.4.2.

#### 4.4.2.2 Fold-rise

For the subjects in the immunogenicity subset, the fold increase will be calculated at any time point (D7, D14, D60, six months …) by dividing the titer/concentration at the time point by that at baseline. For the samples assayed for subjects not in the subset, the fold increase at D60 will be calculated as before (titer/concentration at D60 divided by that at baseline).

### 4.4.3 Efficacy

The calculation of vaccine efficacy is shown in detail in Section 5.1.1.2.

### 4.4.4 Derived Other Variables

#### 4.4.4.1 Age for Demographics

The age for Demographics will be computed as follows:

Age (years) = (Date of Vaccination - Date of Birth + 1)/ 365.25, rounding to the first decimal place.

#### 4.4.4.2 Duration of a Subject in the Trial

The duration of a subject in the study will be computed as follows:

Subject duration (days) = Maximum (date of visit, date of term form) • date of visit V01 +1. Duration will be transformed into months, duration in days*12/365.25 rounded to 1st decimal place.

#### 4.4.4.3 Duration of the Trial

The duration of the study will be computed as follows:

Trial duration (days) = Maximum of all subjects (date of visit, date of term form) • minimum for all subjects (date of visit V01) +1. Duration will be transformed into months, duration in days*12/365.25 rounded to 1st decimal place.

#### 4.4.4.4 Person-time of a Subject to the 1st Event

The person-time of a subject to the 1st event will be computed as follows:

- For subjects experiencing a primary CDI event, person time (MITT) = case start date – 1st vaccination date + 1 day.
- For subjects not having a primary CDI event, person time (MITT) = termination date – 1st vaccination date + 1 day.

Termination date is computed as follows:

- For subjects active in the trial, no termination date, then termination date = data cut-off date.
- For subjects in the trial for more than 3 years with no termination date reported, termination date = last vaccine date + 1095 (3* 365) + 14 (time window) + 1 = last vaccine date + 1110.

# 5 Statistical Methods and Determination of Sample Size

The statistical analyses will be performed under the responsibility of the Sponsor's Biostatistics platform using SAS® Version 9.2 software or later.

Following the recommendation of stopping the study by the independent data monitoring committee (IDMC) at any of the interim analyses or at the final, the data will be locked and ready for analysis. The randomization code will be released. Data in the Clinical Data Management database will be analyzed under the responsibility of the Biostatistics Platform of the Sponsor.

For descriptive purposes, the following statistics will be presented:

**Table 5.1: Descriptive Statistics Produced**

| | | |
|---|---|---|
| **Baseline characteristics and follow-up description** | **Categorical data** | Number of subjects<br>Percentage of subjects |
| | **Continuous data** | Mean, standard deviation, minimum, and maximum |
| **Clinical safety results** | **Categorical data** | Solicited: Number and percentage (95% CIs) of subjects<br>Unsolicited: Number and percentage (95% CIs) of subjects, and number of events |
| **Immunogenicity results** | **Categorical data (fold increase)** | Number and percentage (95% CIs) of subjects |
| | **Continuous data (titer/concentration/data †)** | Log2: Mean and standard deviation<br>Anti-$Log_2$ (work on $Log_2$ distribution, and anti-Log2 applied): Geometric mean, 95% CI of the geometric mean, quartiles, minimum, and maximum<br>Graphical representation by Reverse Cumulative Distribution Curve (RCDC) |

The CI for the single proportion will be calculated using the exact binomial method (Clopper-Pearson method, quoted by Newcombe (12), i.e., using the *F* distribution integral with SAS®.

For immunogenicity, descriptive statistics will be provided for the antibody response concentration against toxins contained in the each treatment group. In general, categorical variables will be summarized and presented by frequency counts, percentages, and CIs. The 95% CIs of point estimates will be calculated using the normal approximation for quantitative data and exact binomial distribution (Clopper-Pearson method) for percentages. For GMCs, 95% CIs of point estimates will be calculated using normal approximation assuming they are log-normally distributed. The CI of the difference in proportion will be calculated using the Wilson Score method without continuity correction. Reverse cumulative distribution curve (RCDC) figures will be provided for the antibody concentrations against each toxin.

## 5.1 Statistical Methods

### 5.1.1 Hypotheses and Statistical Methods for Primary Objective

The primary hypotheses in this study are testing efficacy and futility.

#### 5.1.1.1 Primary Hypothesis for Efficacy

The primary statistical null hypothesis is that there is no greater than a 15% reduction* in the risk of PCR-confirmed cases of CDI among the subjects receiving the vaccine relative to subjects receiving placebo. Formally the trial will test:

$H_0$: VE ≤ 15% *vs.* $H_A$: VE > 15%, where VE=1-$R_v/R_p$ and $R_v$ and $R_p$ are the rates of having a CDI endpoint during follow-up time to the primary or first CDI event in the vaccinated and placebo groups, respectively.

**Note*: There are imperfections in the sensitivity and specificity of PCR that results in an attenuation of VE from that which would be realized under a perfect endpoint assay. We estimate that an observed 15% reduction in PCR-confirmed cases of CDI corresponds to a true VE of 20%; an observed VE of 46% corresponds to an estimated true VE of 60%.

#### 5.1.1.2 Statistical Methods for Efficacy

At the second, third, and the fourth interim analyses and at the final analysis, the primary hypothesis of VE will be tested.

The VE will be estimated by $VE = 1 - R_v / R_p$, where $R_v$ and $R_p$ are the rates of having a CDI endpoint during follow-up. $R_v$ and $R_p$ are estimated by the *events-per-person-time statistic*, i.e., the number of subjects with a confirmed CDI case divided by the total person-time, in the vaccine and placebo groups, respectively, i.e. $R_v = s_v / T_v$ where, $s_v$ and $T_v$ are the number of subjects with a confirmed case and the total person-time in years to the primary or first CDI event in the vaccine group. $R_p = s_p / T_p$ will be estimated by the same method in the placebo group, where $s_p$ and $T_p$ are the number of subjects with a confirmed case and the total person-time in years to the primary or first CDI event in the placebo group.

Note that the efficacy estimate given above, may be restated as $VE = 1 - \frac{T_p}{T_v} \cdot \frac{\theta}{1-\theta}$

where $\theta = s_v / (s_v + s_p)$.

Conditioning on the total number of subjects with a confirmed case in both groups $(s_v + s_p)$, and the assumption that for both groups the number of subjects with a CDI case is a realization from a Poisson distribution (fixed infection rate over time in each group), $s_v$ has a binomial distribution $(\theta, s_v+s_p)$ (13). A (1-α)% CI for $\theta$ may be constructed using the 'exact' method for binomial proportions (12) . The upper limit of the CI for $\theta$ is given by

$ULCI(\theta) = (s_v + 1)[(s_v + 1) + s_p / F_{2(s_v+1),2s_p}(\theta / 2)]^{-1}$, the lower limit for $\theta$ is given by $LLCI(\theta) = s_v[s_v + (s_p + 1)F_{2(s_p+1),2s_v}(\theta / 2)]^{-1}$, where $F_{v1, v2}(\theta/2)$ is the upper $(100\theta/2)$% point of the $F$ distribution with $\theta 1$ and $\theta 2$ degrees of freedom.

Since $\theta/(1-\theta)$ is a strictly increasing function of $\theta$, the lower bound of the (1- $\theta$)% for the VE will be calculated by $LLCI(VE) = 1 - r \cdot \frac{ULCI(\theta)}{1 - ULCI(\theta)}$, and $ULCI(VE) = 1 - r \cdot \frac{LLCI(\theta)}{1 - LLCI(\theta)}$ where $r = T_p / T_v$ (13).

In the case of efficacy testing, the nominal significance levels for the 3 efficacy interim analyses are 0.01, 0.0035, and 0.0032, respectively, as determined by the corresponding proportion of information assessed at each interim analysis (number of new cases at each interim / 250 * 0.025). The remaining alpha (0.0083) after the interim analyses will be used for the final analysis. At any

of the 2nd 3rd, 4th interims and final analyses, if the calculated lower bound of computed CI for VE at its associated significance level is > 15%, the null hypothesis will be rejected and efficacy will be concluded. The analyses will be performed on the modified intent-to-treat (MITT) population. Person-time is calculated as in Section 4.4.4.4.

We note that if interim analyses are performed based on total numbers of cases that are not precisely the same as the aforementioned target milestones, the nominal significance levels at which the interim analyses will be performed and these of the following tests will be adjusted accordingly.

*Note:* analyses of VE will be presented also by age, gender, race/ethnicity and geographical region, see Section 5.1.3.3.

#### 5.1.1.3 Primary Hypothesis for Futility

The tests for futility (non-efficacy) will be conducted testing the null hypothesis (futility) that VE is no less than 46%, which corresponds to a true VE of 60%.

Formally, the following hypothesis will be used:

$H_0$: VE ≥ 46% *vs.* $H_A$: VE < 46%,

where VE=1-$R_v/R_p$ and $R_v$ and $R_p$ are the true rates of having a primary CDI endpoint during the follow-up period in the vaccine and placebo groups, respectively.

#### 5.1.1.4 Primary Statistical Methods for Futility

The primary hypothesis of VE will be tested for futility at the 1st (50 primary cases) and 2nd (100 primary cases) interim analysis. The study will be stopped for futility (non-efficacy) at either of the 2 interim analyses if the computed upper limit of the 2-sided 95% CI for VE is less than the alternative hypothesis value for VE of 46%.

### 5.1.2 Hypotheses and Statistical Methods for Secondary Objectives

#### 5.1.2.1 Secondary Hypotheses for Efficacy

1) The same as the primary hypothesis as in Section 5.1.1.1, the population that will be used in the analyses is the per-protocol population.
2) The same as the primary hypothesis as in Section 5.1.1.1, the population that will be used in the analyses is the subjects who received at least 2 doses of the vaccine.
3) The relative risk of PCR-confirmed CDI cases is not constant over time since enrollment ($H_0$: RR• 1 *vs.* $H_1$: RR=1). This will be tested using a two-sided alpha of 0.05.
4) The secondary statistical null hypothesis is that there is no reduction in the risk of severe PCR-confirmed cases of CDI among the subjects receiving the vaccine relative to subjects receiving placebo. Formally, this hypothesis will test: $H_0$: VE ≤ 0% *vs.* $H_A$: VE > 0%, where VE=1-$R_v/R_p$ and $R_v$ and $R_p$ are the rates of having a severe CDI endpoint during follow-up in the vaccinated and placebo groups, respectively.
5) For the 5th secondary hypothesis, the proportion of subjects in the 3 loose stools categories (frequency of subjects with the maximum loose stools per day in the vaccine and those in the placebo group during the CDI episode will be categorized into 3 categories; 3-5, 6-10 and >10

loose stools) in the vaccine group are equal to those in the placebo group. Formally this hypothesis will test:

$H_0$: $\pi_{iv} = \pi_{ip}$ vs $H_A$: $\pi_{iv} \bullet \pi_{ip}$, $i$ = 1, 2 and 3 is any one of the 3 loose stools category; 3-5, 6-10 and >10 loose stools, where $\pi_{iv}$ and $\pi_{ip}$ are the proportion of subjects in the $i^{th}$ category in the vaccination group and the corresponding proportion in the placebo group, respectively. This hypothesis will be tested using a 2-sided alpha of 0.05.

6) For the 6th secondary hypothesis, there is no reduction in CDI episode/illness duration in subjects who are symptomatic primary PCR-confirmed CDI cases. Formally this hypothesis will test:

$H_0$: $\tau_v \geq \tau_p$ vs $H_A$: $\tau_v < \tau_p$,

where $\tau_v$ and $\tau_p$ are the duration of CDI episode/illness in the vaccine and the placebo groups, respectively, in the symptomatic PCR-confirmed primary CDI case. This hypothesis will be tested using a one-sided alpha of 0.05.

#### 5.1.2.2 Secondary Statistical Methods for Efficacy

- The same statistical method used in Section 5.1.1.2 will be used to evaluate the 1st secondary hypothesis.
- The same statistical method used in Section 5.1.1.2 will be used to evaluate the 2nd secondary hypothesis.
- The statistical null hypothesis for this secondary efficacy analysis is that the magnitude of efficacy does not depend on the time since vaccination, i.e., VE does not wane. This hypothesis will be tested within the framework of relative risk regression models for censored failure time data (14). In this framework, the time from randomization to occurrence of CDI cases will be considered the failure times. For subjects who have not had a clinical CDI endpoint, their time will be considered to be censored at the time of their last follow-up. A relative risk regression model will be fit to these data to estimate the relative risk of CDI case occurrence among vaccinees relative to that among placebo recipients. Because the endpoint rate is low, this estimated relative risk will be nearly identical to 1 minus the VE estimated by the standard Poisson methodology used in the primary analysis of efficacy (described in Section 5.1.1.2). An interaction term between time since randomization and the randomization group indicator will then be statistically tested (at the standard 1-sided Type I error rate of 0.025) for inclusion in the model as a means of formally testing for a time trend in VE that is indicative of waning. A statistically significant interaction term will provide evidence that the risk for the CDI endpoint of vaccinees relative to that for placebo recipients is larger at later follow-up times than at earlier follow-up times. Since VE is formally 1 minus the relative risk, such a result indicates that VE wanes over time. If this formal test for waning VE is significant, explanatory analyses will be performed to examine the nature and extent to which waning occurs.
- The same as the primary hypothesis as in Section 5.1.1.2. The estimated VE will be calculated for subjects who had severe CDI cases, if the calculated lower bound of VE is > 0% the null hypothesis will be rejected and efficacy against the severe cases will be concluded.

95% CI of the VE will be calculated and presented in each of the previous analyses.

- For the 5th secondary hypothesis, the frequency of subjects with the maximum loose stools per day in the vaccine and those in the placebo group during the CDI episode will be categorized into 3 categories; 3-5, 6-10 and >10 loose stools. Comparison of the proportions of the subject in the 3 categories in the vaccine and placebo groups will be made using Fisher's exact test, using 2-sided alpha of 0.05. The following hypothesis will be tested: $H_0$: $\pi_{iv} = \pi_{ip}$ vs $H_A$: $\pi_{iv} \bullet \pi_{ip}$, $i$ = 1, 2 and 3 is any one of the 3 loose stools categories; 3-5, 6-10 and >10, where $\pi_{iv}$ and $\pi_{ip}$ are the proportion of subjects in the $i^{th}$ category in the vaccination group and the corresponding proportion in the placebo group, respectively.
- For the 6th secondary hypothesis, comparison of the CDI episode/illness duration in vaccine and placebo groups will be made using Log Rank Test. The duration of each CDI episode/illness in the vaccine and the placebo groups will be collected and their distributions will be compared using the Log Rank Test using a one-sided alpha of 0.05. The following hypothesis will be tested: $H_0$: $\tau_v \geq \tau_p$ vs $H_A$: $\tau_v < \tau_p$ where $\tau_v$ and $\tau_p$ are the CDI episode/illness duration in the vaccine and placebo group, respectively, in the symptomatic PCR-confirmed CDI cases. If there is a significant reduction in CDI episode/illness duration in the vaccine group, the null hypothesis will be rejected.

#### 5.1.2.3 Secondary Hypotheses for Immunogenicity

No hypotheses to be tested.

#### 5.1.2.4 Secondary Objectives for Immunogenicity

To describe the immunogenicity to toxin A and toxin B:

- in the subset (1,650) of subjects and in subjects with confirmed CDI cases at D0 and D60 (± 14 days)

and

- in the subset (1,650) of subjects at D14 (+3 days), D30 (-3 days to +7 days), and every 6 months up to 3.0 years after the third injection

#### 5.1.2.5 Secondary Statistical Methods for Immunogenicity

For the subset of 1,650 subjects and for subjects with confirmed CDI case at D0 and D60 and for toxin A and B:

- Distribution of TNA and ELISA
- Proportion with a ≥ 4-fold increase in TNA titer and ELISA concentration compared to baseline
- GMTs and GMCs
- RCDCs

For GMTs and GMCs, 95% CIs of point estimates will be calculated using normal approximation assuming antibody titers are log normally distributed. The 95% CI will be calculated as follows $2^{(\bar{x} \pm t_{\cdot /2,n} S_x)}$,

where $2^{(\bar{x})}$ is the GMT, $\bar{x} = \frac{1}{N} \bullet \log_2(x)$, $\log_2(x)$ is the log base 2 of the observed titer, N is the total observations in the vaccination group, $S_{\bar{x}}$ is the standard error, $t_{\bullet/2}$ is the 100(1-• /2) percentile of the standard *t* distribution with the appropriate degrees of freedom *n* and • =0.05.

For proportions of subjects achieving a 4-fold increase compared to baseline, 95% CIs of point estimates will be calculated, assuming that proportions follow a binomial distribution, using Exact binomial methodology (Clopper-Pearson).

#### 5.1.2.6 Secondary Objectives for Safety

To describe the safety profile of all subjects who receive at least 1 injection.

#### 5.1.2.7 Secondary Statistical Methods for Safety

The number and percentage (with 2-sided 95% CIs) of subjects in each group with any of the following will be computed:

- Unsolicited AEs occurring within 30 minutes of each injection
- Solicited injection site reactions occurring within 6 days after the day of each injection (D0 to D6) for subjects in the reactogenicity subset, by intensity, time to onset and number of days of occurrence, will be presented.
- Solicited systemic reactions occurring within 6 days after the day of each injection (D0 to D6) for subjects in the reactogenicity subset, by intensity, time to onset and number of days of occurrence, will be presented.
- Unsolicited AEs and ARs occurring within 30 days after each injection by SOC and preferred term
- All SAEs and related that occur through 6 months after the last injection will be described by nature (MedDRA system organ class and preferred term), seriousness, and outcome.
- All SAEs through the end of the follow-up period

95% CI will be calculated using the exact binomial distribution for percentages (Clopper-Pearson).

### 5.1.3 Hypotheses and Statistical Methods for Exploratory Objectives

#### 5.1.3.1 [redacted]

[redacted]

#### 5.1.3.2 [redacted]

[redacted]

- [redacted]

**5.1.3.3** [redacted]

- [redacted]

- [redacted]

**Table 5.2:** [redacted]

| | | | |
|---|---|---|---|
| [redacted] | | [redacted] | |
| | | [redacted] | [redacted] |

**5.1.3.4** [redacted]

**5.1.3.5** [redacted]

(18).

**5.1.3.6**

**5.1.3.7**

**5.1.3.8**

.

**5.1.3.9**

## 5.2 Analysis Sets

### 5.2.1 Modified Intent-To-Treat Analysis Set

The modified intent-to-treat (MITT) consists of all subjects who received at least 1 injection; subjects will be analyzed according to the group they were randomized to.

*Note:* The MITT will include subjects in Risk Stratum 2 who received at least 1 injection and whose surgery is delayed or cancelled.

### 5.2.2 Per-Protocol Efficacy Analysis Set

The per-protocol (PP) efficacy analysis set is a subset of the MITT. The subjects presenting with at least 1 of the following relevant protocol deviations will be excluded from the PP efficacy:

- Subject did not meet all protocol-specified inclusion criteria or met at least 1 of the protocol-specified exclusion criteria, except for a subject included for an in-patient hospitalization for a planned surgical procedure and the surgery was postponed beyond 60 days
- Subject did not receive any vaccine or subject did not complete the vaccination schedule
- Subject received a different vaccine other than the one that he / she was randomized to receive
- Preparation and/or administration of vaccine was not done as per protocol
- Subject did not receive vaccine in the proper time window according to the protocol
- Subject received a protocol-restricted therapy / medication / vaccine affecting the subject's immune response

In the event of a local or national immunization program with a pandemic influenza vaccine, subjects who receive 1 or more doses of a pandemic influenza vaccine at any time during the trial will not be withdrawn from the trial.

**Per-Protocol Immunogenicity Analyses Set:**

The per-protocol (PP) immunogenicity analysis set is a subset of the MITT. The subjects presenting with at least one of the following relevant protocol deviations will be excluded from the PP immunogenicity:

- Subject did not meet all protocol-specified inclusion criteria or met at least one of the protocol-specified exclusion criteria
- Subject did not receive vaccine or subject did not complete the vaccination schedule
- Subject received different vaccine other than the one that he / she was randomized to receive
- Preparation and / or administration of vaccine was not done as per protocol
- Subject did not receive vaccine in the proper time window according to the protocol
- Subject did not provide post-dose serology sample in the proper time window or a post-dose serology sample was not drawn
- Subject received a protocol-restricted drug or vaccine affects his/her immune response
- Subject's serology sample did not produce a valid test result

In the event of a local or national immunization program with a pandemic influenza vaccine, subjects who receive one or more doses of a pandemic influenza vaccine at any time during the trial will not be withdrawn from the trial.

### 5.2.3 Safety Analysis Set

The safety analysis set (SafAS) is defined as those subjects who have received any of the study vaccines or placebo. All subjects will have their safety analyzed according to the vaccine they actually received. If the vaccine received by a subject does not correspond to any study group, the subject will be excluded from the SafAS.

### 5.2.4 Other Analysis Set

The full analysis set (FAS) is defined as the subset of subjects who received at least one dose of the study vaccine and had at least one valid result for post-vaccination blood sample.

## 5.3 Populations Used in Analyses

- **Primary Hypothesis:**

Subjects in the MITT will be used in the primary efficacy hypothesis.

- **Secondary Hypotheses**

1) Efficacy:
   - Subjects in the PP efficacy analyses set will be used in the 1st secondary efficacy hypothesis.
   - Subjects in the MITT will be used in the rest of secondary efficacy hypotheses.

2) Immunogenicity:
   - All subjects in the FAS who participated in the immunogenicity subset and subjects with PCR-confirmed CDI case will be included in the immunogenicity analyses.
   - The same immunogenicity analyses will be performed using the PP immunogenicity subjects.
3) Safety:

   Subjects in the safety analyses set will be used in the secondary safety analyses.

- [redacted]

1) [redacted]

## 5.4 Handling of Missing Data and Outliers

### 5.4.1 Safety

No replacements will be done.

In all subject listings, partial and missing data will be clearly indicated as missing.

### 5.4.2 Immunogenicity

For the computation of GMT/GMC, a titer/concentration reported as <LLOQ will be converted to a value of 0.5 LLOQ.

For calculating a fold-rise, < LLOQ will be converted to 0.5 LLOQ for a numerator and < LLOQ will be converted to LLOQ for a denominator when only one of either the numerator or denominator is < LLOQ. If both the numerator and denominator are < LLOQ, then both will be

converted in the same way (i.e., fold-rise = 1.0). For any calculations, a titer reported as > upper limit of quantitation (ULOQ) will be converted to a value of ULOQ.

No replacements will be done.

In all subject listings, partial and missing data will be clearly indicated as missing.

### 5.4.3 Efficacy

The method of multiple imputations will be used where missing data will be imputed under a Missing At Random (MAR) assumption. The primary analytic results will be deemed robust if the conclusions are unchanged across a reasonable range of departures from the MAR assumption.

## 5.5 Interim/Preliminary Analysis

The study is designed as an event-driven, group sequential protocol with 4 interim analyses at 50, 100, 135, 167 primary clinical endpoints (i.e., PCR-confirmed primary CDI episodes), and a final analysis. The first interim will assess futility only after observing 50 primary CDI case. The second interim analysis will assess futility and efficacy after observing 100 primary CDI cases. The third and fourth interim analysis will assess efficacy and occur when 135 and 167 primary clinical endpoints are observed, respectively. A final analysis will be done when the trial stops.

In case of observing the milestone number of symptomatic PCR-confirmed primary CDI cases to trigger an interim analysis, an interim analysis of the futility and efficacy of the vaccine relative to placebo for the primary futility and efficacy endpoint will be reviewed by the IDMC. The IDMC may recommend stopping the trial based on the statistical significance guidelines in the IDMC charter for early stopping.

In addition to the statistical guidelines for early stopping, the IDMC may provide an additional guideline for early stopping based on the average duration of follow-up from enrollment by study participants. Specifically, IDMC may recommend early stopping of the trial for purpose of efficacy only if the statistical test criterion is met and the median duration of follow-up is no less than 12 months after injection. Although simulation studies indicate that the average duration of follow-up will be no less than 12 months at the time that a sequential trial boundary is achieved with very high probability, this parameter will be monitored closely and an interim analysis will be delayed to ensure that the duration of follow-up criterion, median duration of follow-up is no less than 12 months after injection, is met before the interim statistical analysis for efficacy is performed. In this case, a small adjustment to the nominal significance level at which the interim analysis is performed will be used based on the alpha-spending function used in Section 5.1.1.2.

Refer to the IDMC charter on the steps how the IDMC recommendations are made.

If the IDMC recommendation is to change the course of the trial (i.e., stop the trial for futility or efficacy), the Sanofi Pasteur unblinded statistician (SPUS) will validate the IDMC decision. The SPUS will independently calculate VE and the associated CI for the primary efficacy objective and compare it to the independent statistician-generated values. If the SPUS finds no discrepancy with the independent statistician values, this will be confirmed to the Sponsor representative. If the SPUS identifies a discrepancy with the independent statistician values, the SPUS will inform Sponsor representative of the discrepancy, and contact the independent statistician to investigate the discrepancy and resolve it. Sanofi Pasteur unblinded review committee not directly involved

in the conduct of the study will evaluate the unblinded data and make a recommendation to Sanofi Pasteur senior management. Senior management will make a final decision as to whether there is a agreement or not with the IDMC recommendation. Sanofi Pasteur senior management will document their decision and inform the IDMC, Center for Biologics Evaluation and Research (CBER) and the Institutional Review Board (IRB) with their decision.

## 5.6 Handling of Multiplicity

- **Multiplicity for Primary Objective**

This study is designed as a group sequential design with 4 interim analyses and one final analysis. The multiplicity adjustment for primary objectives can be found in Section 5.1.1.2.

- **Multiplicity for Secondary Objectives**

The first two secondary objectives are testing the same hypotheses as primary hypothesis in the sub-population of MITT, which further categorize the vaccine effect on population who received two or three doses. The fourth objective tests whether the vaccine has any effect in reducing the risk of severe PCR-confirmed cases of CDI in the MITT population. The fifth and sixth secondary objectives test the vaccine effect in reducing loose stool and duration among the primary PCR-confirmed CDI cases. All those secondary objectives further describe the effect of the tested vaccine on different subpopulation of MITT. Therefore, no multiplicity adjustment proposed for the secondary hypotheses testing.

## 5.7 Determination of Sample Size and Power Calculation

A total of 16,500 subjects are planned to be enrolled:

*C. difficile* Vaccine Group: n = 11,000

Placebo Group: n = 5,500

Stratification will be based on the 2 major inclusion criteria and will be defined as follows:

Risk Stratum 1:

- Has had at least 2 hospital stays, each lasting at least ≥ 24 hours, in the 12 months before enrollment

and

- Has received systemic (not topical) antibiotics in the 12 months before enrollment

or

Risk Stratum 2:

- Is anticipated to have an in-patient hospitalization for a planned surgical procedure within 60 days of enrollment and the impending hospital stay is planned to be ≥ 72 hours (see also Inclusion Criteria in the protocol).

This stratification, together with geographical region-blocks randomization and fixed block size of 3, will ensure the planned 2:1 balance between vaccine and placebo groups during the entire course of study enrollment. The relative numbers of subjects enrolled into each stratum will be

monitored during both enrollment stages of the trial. If the fraction enrolled to any 1 stratum drops below 30%, then the study team will review the operational approaches to recruitment and recommend changes to the approach that will increase the relative rate of enrollment to the smaller group.

[redacted]

## 5.8 Data Review for Statistical Purposes

Review of the data is anticipated through the data review process led by data management before database lock. Any review of the data will include a statistical review, for completeness and data range assessments.

Reviews will be conducted on the blinded data.

## 5.9 Changes in the Conduct of the Trial or Planned Analyses

This SAP complies with the International Conference on Harmonisation (ICH) guidance and relevant Food and Drug Administration (FDA) guidance. It is based on Version 7.0 of the study protocol dated 14 February 2017. If the protocol is subsequently amended, this SAP may be amended as well. Should the SAP and the protocol be inconsistent with respect to the planned analyses, the language of the SAP is governing.

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