A PHASE 3, PLACEBO-CONTROLLED, RANDOMIZED, OBSERVER-BLINDED STUDY TO EVALUATE THE EFFICACY, SAFETY, AND TOLERABILITY OF A *CLOSTRIDIUM DIFFICILE* VACCINE IN ADULTS 50 YEARS OF AGE AND OLDER

Investigational Product Number:	PF-06425090
Investigational Product Name:	*Clostridium difficile* Vaccine
United States (US) Investigational New Drug (IND) Number:	CCI
European Clinical Trials Database (EudraCT) Number:	2016-003866-14
Protocol Number:	B5091007
Phase:	3

Document History

Document	Version Date	Summary of Changes
Original protocol	28 October 2016	Not applicable (N/A)
Protocol amendment 1	26 January 2017	Following regulatory consultation: • Rationalized objectives and endpoints. • Clarified case definitions. • Revised permissible timing for administration of nonadjuvanted seasonal or pandemic influenza vaccine. • Added a Month 12 telephone contact for collection of serious adverse events. • Changed description of diagnostic methodology for detection of toxin. • Added safety reporting requirements for studies employing medical devices. • Updated statistical methodology to reflect changes in objectives and endpoints. • Made minor editorial updates.
Protocol amendment 2	03 January 2019	In order to control overall Type I error and Type I error for the evaluation of the secondary objectives: • Revised secondary CCI CCI objectives and endpoints. Following blinded case count review: • Revised sample size.

		Additionally: • Refined the parameters collected for and included in the healthcare resource utilization score. • Defined the mITT-1 population. • Included analyses of the primary endpoints for the m-ITT populations for cases occurring 14 days after the second and third doses. • Made editorial updates to the Data Analysis/Statistical Methods section. • Incorporated a protocol administrative change to the medical device complaint reporting procedure. • Incorporated a protocol administrative change to clarify that Visit 6 can be conducted in person. • Incorporated France- and Japan-specific appendices.
Protocol Amendment 3	06 January 2020	• Added the annual retention visit. • Added the requirement to reconsent subjects at the additional visit. • Extended the study duration.
Protocol Amendment 4	28 Sept. 2020	• Added the definition of an outpatient visit to inclusion criterion 4. • Clarified systemic antibiotic use for the purposes of inclusion criterion 4. • Added a reminder every 2 weeks for all subjects to collect a stool sample should a diarrheal episode occur. • Clarified the requirement to capture major protocol deviations.

		• Clarified that if an SAE is reported after subject Visit 6, all the requirements will apply, including the respect of the reporting timelines as if the SAE occurred within the reporting period. • Adjusted the assumption for vaccine efficacy and the total cases required for the study, introduced 2 additional interim analyses, and updated the statistical design operating characteristics accordingly.

This amendment incorporates all revisions to date, including amendments made at the request of country health authorities and institutional review boards (IRBs)/ethics committees (ECs).

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Indication

Pfizer's investigational *Clostridium difficile* vaccine (PF-06425090) is a prophylactic vaccine that is currently being investigated for the prevention of primary *C difficile* infection (CDI) in adults 50 years of age and older.

Objectives and Endpoints

Primary Efficacy Objective(s):	**Primary Efficacy Endpoint(s):**

• To demonstrate that Pfizer's *C difficile* vaccine is effective in reducing the incidence of a first primary episode of CDI (see case definition 1).[a]	• CDI incidence per 1000 person-years of follow-up, assessed during up to 2 time periods (each analysis will be performed only if the preceding one was successful):
	  • After receipt of the third dose of investigational product onwards.
	  • After receipt of the second dose of investigational product onwards.

Primary Safety Objective(s):	**Primary Safety Endpoint(s):**
• To evaluate the safety profile of Pfizer's *C difficile* vaccine as measured by the percentage of subjects reporting local reactions and systemic events, adverse events (AEs), and serious adverse events (SAEs).	• Local reactions (pain, erythema, and induration), as self-reported on electronic diaries (e-diaries) for up to 7 days following each dose of investigational product. • Systemic events (fever, vomiting, headache, fatigue, new or worsening muscle pain, and new or worsening joint pain), as self-reported on e-diaries for up to 7 days following each dose of investigational product. • Nonserious AEs from signing of the informed consent document (ICD) to 1 month after receipt of the third dose of investigational product. • SAEs from signing of the ICD to 6 months after receipt of the third dose of investigational product.

a. Each subject may contribute only once to the primary endpoint.

Study Design

This is a Phase 3, placebo-controlled, randomized, observer-blinded study to evaluate the efficacy, safety, and tolerability of aluminum hydroxide (AlOH)-containing *C difficile* vaccine (200 µg total toxoid) administered as a 3-dose regimen at Months 0, 1, and 6 in adults 50 years of age and older.

Subjects will be randomly assigned in parallel in a 1:1 ratio to receive *C difficile* vaccine (200 µg total toxoid) or placebo (saline).

This is an event-driven study with a total target of 66 first primary CDI cases (meeting case definition 1 in the per-protocol-3 population); the total enrollment number may vary depending on the incidence rate of the primary endpoint, true underlying vaccine efficacy (VE), and potential early stop for efficacy or futility.

Investigational Products

***Clostridium difficile* Vaccine**

The investigational *C difficile* vaccine is toxoid based. C *difficile* toxin A and toxin B are inactivated by a combination of genetic mutations and chemical treatments. The vaccine is provided as a sterile lyophilized powder in a dosage strength of 200 µg/dose (total for toxoids A and B). The vaccine will be reconstituted with AlOH diluent immediately before use as instructed in the investigational product (IP) manual. The AlOH diluent is supplied as a 1-mg aluminum/mL (as AlOH) liquid suspension.

Placebo

The placebo will consist of a sterile normal saline solution for injection (0.9% sodium chloride injection, in a 0.5-mL dose) and will be provided by the sponsor to each study site.

Statistical Methods

The VE is defined as VE = 100 × (1 – IRR), where IRR is the infection rate ratio, the calculated ratio of first primary CDI incidence between the *C difficile* vaccine group and the placebo group.

Three (3) formal interim analyses are planned to check both efficacy and futility after 30, 40 and 50 cases of first primary CDI have occurred from 14 days after receipt of the third dose of investigational product. Only the primary efficacy endpoints will be examined during these interim analyses.

With 30 cases of first primary CDI at the first planned interim analysis, if there are 6 or fewer cases observed in the *C difficile* vaccine group, the null hypothesis (H_{03}) will be rejected, the *C difficile* vaccine will be deemed efficacious, and a full analysis of the data will be conducted. If there are 11 or more cases observed in the *C difficile* vaccine group, futility may be declared, and the study may be terminated.

With 40 cases of first primary CDI at the second planned interim analysis, if there are 9 or fewer cases observed in the *C difficile* vaccine group, the null hypothesis (H_{03}) will be rejected, the *C difficile* vaccine will be deemed efficacious, and a full analysis of the data may be conducted. If there are 13 or more cases observed in the *C difficile* vaccine group, futility may be declared, and the study may be terminated.

With 50 cases of first primary CDI at the third planned interim analysis, if there are 13 or fewer cases observed in the *C difficile* vaccine group, the null hypothesis (H_{03}) will be rejected, the *C difficile* vaccine will be deemed efficacious, and a full analysis of the data may be conducted. If there are 16 or more cases observed in the *C difficile* vaccine group, futility may be declared, and the study may be terminated.

If the study continues after 3 interim analyses, for the final analysis, the primary objective will have been met if there are 20 or fewer cases observed in the *C difficile* vaccine group out of a total of 66 first primary cases from 14 days after receipt of the third dose of investigational product onwards.

AEs will be summarized using 3-tier methodology. Local reactions and systemic events will be summarized.

SCHEDULE OF ACTIVITIES

The schedule of activities table provides an overview of the protocol visits and procedures. Refer to the Study Procedures and Assessments sections of the protocol for detailed information on each procedure and assessment required for compliance with the protocol.

The investigator may schedule visits (unplanned visits) in addition to those listed on the schedule of activities table, in order to conduct evaluations or assessments required to protect the well-being of the subject.

Visit ID	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Annual Retention Visit**	**Visit 7**	**At Time of Potential CDI Case**
Visit Description	**Month 0 (Vax 1)**	**Month 1 (Vax 2)**	**Month 2**	**Month 6 (Vax 3)**	**Month 7**	**Month 12**		**End of Study**	
Visit Window (Days)	**1**	**28-42 Days After Visit 1**	**28-42 Days After Visit 2**	**140-168 Days After Visit 2**	**28-42 Days After Visit 4**	**165-195 Days After Visit 4**	**Every 280-392 Days After Visit 5**[a]	**Not Applicable**	
Informed consent[b]	X						X[c]		
Demography[b]	X								
Clinical assessment, including medical history[b]	X								
Record nonstudy vaccinations[d]	X	X	X	X	X				
Record specified concomitant medications									X
Measure and record height and weight[b]	X								
Oral temperature[b]	X	X		X					
Urine pregnancy test (females of childbearing potential)[b]	X	X		X					
Discuss contraceptive use[d]	X	X	X	X					
Confirm eligibility[d]	X	X	X	X					
Review temporary delay criteria[b]	X	X		X					
Randomization[b]	X								
Blood draw for immunogenicity assessment[d]	X		X		X				X[e]

Visit ID	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Annual Retention Visit**	**Visit 7**	**At Time of Potential CDI Case**
Visit Description	**Month 0 (Vax 1)**	**Month 1 (Vax 2)**	**Month 2**	**Month 6 (Vax 3)**	**Month 7**	**Month 12**		**End of Study**	
Visit Window (Days)	**1**	**28-42 Days After Visit 1**	**28-42 Days After Visit 2**	**140-168 Days After Visit 2**	**28-42 Days After Visit 4**	**165-195 Days After Visit 4**	**Every 280-392 Days After Visit 5[a]**	**Not Applicable**	
Vaccination	X	X		X					
Postvaccination observation (at least 30 minutes) and AE assessment	X	X		X					
Review stool sample collection and shipping instructions with the subject and ensure understanding	X	X	X	X	X	X	X		
Issue or send a stool collection kit and shipping box, and provide instructions on their use, as required	X	X	X	X	X	X	X		
Issue measuring device, and thermometer and provide instructions on their use, as required	X	X		X					
Review subject communication methods (including for e-diary completion), assist the subject with downloading the app, or issue provisioned device, if required	X	X	X	X	X	X	X		
Record AEs	X	X	X	X	X[f]				X[f]
Record SAEs	X	X	X	X	X	X			
Capture major protocol violations		X	X	X	X	X	X	X	X
Telephone contact visit						X[g]		X	X
Electronic reminders		←	—	—	—	—	—	→	
Telephone contact for subjects unresponsive to electronic reminders		←	—	—	—	—	—	→	
Provision of stool sample									X
Completion of diarrheal episode questionnaire									X

Visit ID	**Visit 1**	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**	**Annual Retention Visit**	**Visit 7**	**At Time of Potential CDI Case**
Visit Description	**Month 0 (Vax 1)**	**Month 1 (Vax 2)**	**Month 2**	**Month 6 (Vax 3)**	**Month 7**	**Month 12**		**End of Study**	
Visit Window (Days)	**1**	**28-42 Days After Visit 1**	**28-42 Days After Visit 2**	**140-168 Days After Visit 2**	**28-42 Days After Visit 4**	**165-195 Days After Visit 4**	**Every 280-392 Days After Visit 5[a]**	**Not Applicable**	
Subject completes e-diary	X	X		X					
Review e-diary data		←	—	—	—	→			

Abbreviations: CDI = *Clostridium difficile* infection; e-diary = electronic diary; Vax = vaccination; → = ongoing/continuous event.

a. Following approval of Amendment 3, subjects already enrolled in the study should have an annual retention visit arranged at the earliest opportunity, and then every 280 to 392 days thereafter.
b. Prior to vaccination.
c. At the first annual retention visit or potential CDI visit, whichever occurs first.
d. Prior to vaccination, if at a vaccination visit.
e. Optimally within 48 hours of the third unformed stool (Bristol stool chart types 5-7).
f. Any AEs occurring up to 48 hours after each blood draw must be recorded.
g. Visit is intended to be conducted by telephone but, if desired, may be conducted in person.

1. INTRODUCTION

1.1. Mechanism of Action/Indication

Pfizer's investigational *Clostridium difficile* vaccine (PF-06425090) is a prophylactic vaccine that is currently being investigated for the prevention of primary *C difficile* infection (CDI) in adults 50 years of age and older.

1.2. Background and Rationale

1.2.1. *Clostridium difficile* Disease Background

C difficile, a gram-positive anaerobic, spore-forming bacillus, is the main cause of nosocomial infectious diarrhea in industrialized countries.[1,2,3,4,5] It accounts for 20% to 30% of cases of antibiotic-associated diarrhea and is the most commonly recognized cause of infectious diarrhea in healthcare settings.[6] *C difficile* is carried in approximately 3% of healthy adults and approximately 16% to 35% of hospital inpatients; among the latter the rate increases with exposure to antibiotics.[7] As many as 50% or more of hospital patients colonized by *C difficile* are asymptomatic carriers.[6]

C difficile can produce 3 toxins, toxin A (TcdA), toxin B (TcdB), and binary toxin.[8] TcdA and TcdB are the principal virulence factors for CDI,[9] causing severe inflammation in the bowel.[10,11] The spectrum of CDI ranges from asymptomatic colonization and mild self-limiting diarrhea, to more serious complications such as severe diarrhea, pseudomembranous colitis, toxic megacolon, intestinal perforation, and death.[2,12]

Individuals with mild to moderate CDI may experience diarrhea 3 or more times a day for 2 or more days, along with mild abdominal cramping and tenderness. However, individuals with a severe infection may experience watery diarrhea 10 to 15 times a day, abdominal cramping and pain that may be severe, fever, blood or pus in the stool, nausea, dehydration, loss of appetite, weight loss, swollen abdomen, kidney failure, and increased white blood cell count.[13]

The main risk factors for an initial episode of CDI are antibiotic therapy, prolonged hospitalization, and severe comorbidities. Older adults (>65 years of age) are at an increased risk for CDI, particularly when exposed to healthcare settings.[11,14,15,16,17] Although most patients experiencing a first episode of CDI respond well to standard antibiotic treatment, approximately 15% to 35% of patients suffer from at least 1 recurrence.[11,18,19] Additional risk factors for recurrence include failure to mount an immune response to TcdA and/or TcdB and infection with a hypervirulent strain.[20,21]

Increased incidence and severity of the disease with associated complications, colectomy rates, and mortality have been observed over the last 10 to 20 years.[22,23,24] In Europe, increased mortality, prolonged length of hospital stay, and incremental cost of hospitalization in association with CDI have been reported.[25,26] A systematic review published in 2012 reported 30-day mortality estimates in Europe ranging from 6.8% to 42%.[26] In a surveillance study conducted in the United States, approximately 453,000 cases of CDI and approximately 29,000 deaths were reported in 2011.[27]

Based on data reported from acute care facilities, the cost of caring for patients experiencing CDI in the United States ranges from $1.1 to $4.8 billion annually.[28,29]

In the last decade, the numbers and severity of *C difficile* iatrogenic outbreaks in hospitals and nursing homes have increased.[27,28,30] Key factors in this escalation include emergence of hypervirulent pathogenic strains such as BI/NAP1/027,[31,32,33] increased use of antibiotics,[34] improved detection methods, and increased exposure to spores in healthcare facilities.[35]

Metronidazole has been recommended as initial therapy since the late 1990s and continues to be the first choice for all but seriously ill patients and those with complicated or fulminant infections or multiple recurrences of CDI, for whom vancomycin is recommended.[6,21,36] Fidaxomicin has been developed more recently and has been shown to be noninferior to vancomycin for treatment of CDI. In addition, treatment with fidaxomicin was associated with a significantly lower rate of recurrence than was treatment with vancomycin.[19] European guidelines for treatment of CDI include fidaxomicin as an option for treatment of recurrence.[21] Fecal transplantation has also been shown to be effective in some patients for the treatment of multiple recurrences of CDI.[37,38] However, regulatory oversight of this method remains in development,[39] and, therefore, a highly effective noninvasive treatment for complicated CDI does not exist.

To date, the only measures available to help prevent CDI are behavioral ones such as encouraging appropriate use of antimicrobials, use of contact precautions, and cleaning and disinfection of equipment and the environment.[40]

1.2.2. *Clostridium difficile* Vaccine Development Rationale

It is well established that humoral immune responses to *C difficile* toxins play a significant role in preventing a more severe outcome or a recurrence of the disease in humans. Several clinical studies suggest a correlation between high serum concentrations of antitoxin A and B immunoglobulin G (IgG) (as measured by enzyme-linked immunosorbent assay [ELISA]) and protection from CDI or recurrence after primary CDI.[20,41,42,43] Preclinical studies have shown that active immunization with inactivated toxins ("toxoids") and passive immunization with antitoxin antibodies protect animals from lethal challenge.[44,45,46] Furthermore, a Phase 2 trial with monoclonal antibodies (mAbs) designed to neutralize TcdA and TcdB provides clinical evidence supporting a role for antitoxin antibodies in the prevention of CDI recurrences.[47] Systemic administration of a combination of 2 neutralizing human mAbs against TcdA and TcdB was associated with fewer recurrences than placebo (7% versus 25% recurrences, respectively; $p<0.001$). These results were confirmed in 2 Phase 3 studies. In both studies, results showed that the rate of recurrence of CDI was significantly lower when the mAb against TcdB (bezlotoxumab) was administered, whether on its own or in combination with the mAb against TcdA (actoxumab), compared with placebo.[48] Finally, another group demonstrated that a combination of 2 mAbs directed against different epitopes from the binding domain of TcdA was significantly more potent in neutralizing the toxin in vitro than a single mAb.[49] Furthermore, while the TcdA and TcdB amino acid sequences are generally well conserved within the same sequence types, there is high variation between sequence types,[50] indicating that a polyclonal antibody approach, such as elicited by active immunization with *C difficile* toxoids, may be more efficacious to

prevent primary and recurrent CDI. Taken together, these findings provide the rationale for the development of a *C difficile* toxoid-based vaccine to prevent CDI that includes both TcdA and TcdB. Pfizer therefore is developing a bivalent vaccine composed of both toxoids to elicit broad antitoxin immunity against TcdA and TcdB.

To date, there is no approved vaccine to prevent primary or recurrent CDI. There are at least 2 other vaccines in development that target *C difficile* toxins. A toxoid-based vaccine adjuvanted with aluminum hydroxide (AlOH) has been evaluated in Phase 1 and 2 studies,[51,52,53,54] and efficacy is currently being evaluated in a Phase 3 study.[55] The other is a recombinant fusion protein comprising fragments of the receptor-binding domains of TcdA and TcdB separated by a 4–amino acid linker;[56] this vaccine has been evaluated in a completed Phase 2 study.[57]

The increasing burden of CDI on patients and on the healthcare system demonstrates that prevention of CDI constitutes a significant unmet medical need.

1.2.3. *Clostridium difficile* Vaccine Candidate

Pfizer's *C difficile* vaccine candidate consists of a 1:1 mixture of *C difficile* toxoids A and B. The toxoids were derived from native toxins by genetic modification to decrease toxin activity, and chemical inactivation prior to final purification and formulation of the drug substance.

1.2.3.1. Nonclinical Development

In nonclinical experiments, Pfizer's *C difficile* candidate vaccine was studied either alone or in combination with AlOH. Using the standard hamster *C difficile* disease model, vaccine formulations with and without AlOH demonstrated a survival benefit, providing at least 90% protection from a lethal challenge with *C difficile* spores in the immunized hamsters. In addition, pooled sera obtained from hamsters immunized with the *C difficile* vaccine formulated with AlOH neutralized secreted toxins from *C difficile* isolates representing diverse ribotypes/pulsed-field gel electrophoresis (PFGE) types, including hypervirulent strains, and covering >67% and >70% of the circulating strains in the United States and Europe, respectively. Furthermore, in nonhuman primates, Pfizer's toxoid vaccine formulations with and without AlOH induced robust neutralizing antitoxin antibody responses to both TcdA and TcdB. The nonclinical data generated by Pfizer in rhesus macaques support the use of a 3-dose regimen of the *C difficile* vaccine, with or without AlOH.

1.2.3.2. Clinical Development

The B5091001 first-in-human study was a placebo-controlled, randomized, observer-blinded Phase 1 study that evaluated the safety, tolerability, and immunogenicity of Pfizer's *C difficile* vaccine. Three (3) antigen dose levels (50, 100, and 200 μg) were assessed and administered either alone or in combination with AlOH at Months 0, 1, and 6 to healthy adults 50 to 85 years of age.[58] Overall, the *C difficile* vaccine formulations and dose levels administered were generally well tolerated. Local reactions and systemic events were predominantly mild to moderate, were more common in the 50- to 64-year age cohort, and

comprised mostly injection site pain, headache, and fatigue. Adverse events (AEs) were reported in all vaccine groups, with little difference in the number of subjects between the dose groups and the placebo group or between vaccine formulations. In subjects who received the vaccine formulations, both the toxin A– and toxin B–specific neutralizing antibody geometric mean concentrations increased substantially at 1 month after Dose 2 and after Dose 3 compared to baseline. In the 50- to 64-year age cohort, geometric mean fold rises (GMFRs) in toxin A–specific neutralizing antibodies from baseline at Month 7 ranged from 59.19 to 149.23 in the dose groups compared to 2.47 in the control group. For toxin B–specific neutralizing antibodies, the GMFRs from baseline at Month 7 ranged from 116.67 to 2503.75 in the dose groups compared to 2.48 in the control group. In the 65- to 85-year age cohort, the GMFRs in toxin A–specific neutralizing antibodies from baseline at Month 7 ranged from 42.73 to 254.77 in the dose groups compared to 2.03 in the control group. For toxin B–specific neutralizing antibodies, the GMFRs from baseline at Month 7 ranged from 136.12 to 4922.80 in the dose groups compared to 1.58 in the control group. Potent antitoxin neutralizing responses were still evident in immunized subjects in both age groups at Month 12. Although there was no clear dose-level response pattern, the data suggest that both the antitoxin A– and antitoxin B–specific neutralizing responses were trending higher in the toxoid-only groups compared to the toxoid + AlOH groups. Furthermore, the magnitude of the immune response was similar in the 2 age cohorts.

Based on these data, a Phase 2 study (B5091003) was initiated using a 3-dose regimen (Days 1, 8, and 30) and 2 antigen dose levels (100 and 200 μg) of the toxoids alone reconstituted with sodium chloride diluent. However, recruitment and vaccinations were halted because of the occurrence of 7 cases of Grade 3 injection site redness after Dose 2. In light of this observed tolerability profile, the decision was made to progress development of the AlOH-containing formulation into a second Phase 2 study. This Phase 2 study (B5091009) of the AlOH-containing formulation is ongoing to assess the safety, tolerability, and immunogenicity of 2 antigen dose levels (100 and 200 μg) and two 3-dose regimens (Days 1, 8, and 30 and Months 0, 1, and 6). A prespecified interim analysis of data up to Month 7 from this study demonstrated that the immunogenicity profile following 3 doses administered at Months 0, 1, and 6 was superior to that when the doses were administered at Days 1, 8, and 30. In addition, the 200-μg dose level was more immunogenic than the 100-μg dose level. On this basis, it was decided to progress into Phase 3 development with the 200-μg dose level administered at Months 0, 1, and 6.

The present study (B5091007) is therefore a placebo-controlled, randomized (1:1, vaccine:placebo), observer-blinded, parallel-group study in up to approximately 17,476 subjects 50 years of age or older who have an increased risk of CDI. In the absence of an accepted immunological correlate of protection for CDI, vaccine efficacy (VE) will be determined by comparing the CDI incidence in recipients of the investigational vaccine with those receiving placebo. Any time during the study period that a subject experiences passage of 3 or more unformed stools (Bristol stool chart types 5-7; see Appendix 2)[59] within 24 hours, the subject will be asked to collect the third or subsequent stool for provision to the central laboratory.

Stool specimens will be tested at a central laboratory for presence of the *C difficile* organism and TcdA/TcdB. The study is designed to accrue 66 first primary CDI cases, meeting case definition 1 (Section 2.1.1), occurring from 14 days after the third dose of vaccine.

1.2.3.3. Summary of Benefit-Risk Assessment

C difficile is the main cause of nosocomial infectious diarrhea in industrialized countries.[1,2,3,4,5] It accounts for 20% to 30% of cases of antibiotic-associated diarrhea and is the most commonly recognized cause of infectious diarrhea in healthcare settings.[6] To date, there is no approved vaccine to prevent primary or recurrent CDI. The increasing burden of CDI on patients and on the healthcare system demonstrates that prevention of CDI constitutes a significant unmet medical need.

The B5091001 first-in-human study, which assessed 3 antigen dose levels (50, 100, and 200 µg) administered either alone or in combination with AlOH at Months 0, 1, and 6 to healthy adults 50 to 85 years of age, demonstrated that the *C difficile* vaccine formulations and dose levels administered were generally well tolerated. Local reactions and systemic events were predominantly mild to moderate, and comprised mostly injection site pain, headache, and fatigue. There was little difference in the number of subjects reporting AEs between the dose groups and the placebo group or between vaccine formulations.

The B5091009 Phase 2 study, which assessed 2 antigen dose levels (100 and 200 µg) administered in combination with AlOH at Months 0, 1, and 6 or Days 1, 8, and 30 to healthy adults 65 to 85 years of age, demonstrated that both regimens and both dose levels administered were generally well tolerated. Local reactions were predominantly mild to moderate, with injection site pain being the most frequent manifestation. After Dose 2, local reactogenicity was greater when the vaccine was administered at Day 8 compared to Month 1, particularly for the 200-µg dose level. Systemic events were also predominantly mild to moderate and the incidences of individual events were similar between the placebo group, the 100-µg dose group, and the 200-µg dose group. Within each regimen, the overall AE incidence rates were also similar between the placebo, 100-µg, and 200-µg dose groups. For both regimens, serious adverse events (SAEs) were numerically higher in the 100-µg and 200-µg dose groups than in the placebo group. However, there was no pattern to these events and no safety concern was identified. Both studied dose levels resulted in substantial neutralizing antitoxin A and B titers, with the immunogenicity profile following 3 doses administered at Months 0, 1, and 6 being preferred. In addition, the 200-µg dose level was more immunogenic than the 100 µg dose level.

Pfizer considers the available information from Studies B5091001 and B5091009 with PF-06425090 to support a favorable benefit-risk profile for studies administering 3 doses of the *C difficile* vaccine at dose levels up to 200 µg formulated with AlOH, as a potential prevention against CDI.

Additional information for this compound may be found in the single reference safety document (SRSD), which for this study is the investigator's brochure (IB).

2. STUDY OBJECTIVES AND ENDPOINTS

Primary Efficacy Objective(s):		**Primary Efficacy Endpoint(s):**

• To demonstrate that Pfizer's *C difficile* vaccine is effective in reducing the incidence of a first primary episode of CDI (see case definition 1).[a]		• CDI incidence per 1000 person-years of follow-up, assessed during up to 2 time periods (each analysis will be performed only if the preceding one was successful):
		  • After receipt of the third dose of investigational product onwards.
		  • After receipt of the second dose of investigational product onwards.

Primary Safety Objective(s):		**Primary Safety Endpoint(s):**
• To evaluate the safety profile of Pfizer's *C difficile* vaccine as measured by the percentage of subjects reporting local reactions and systemic events, AEs, and SAEs.		• Local reactions (pain, erythema, and induration), as self-reported on electronic diaries (e-diaries) for up to 7 days following each dose of investigational product. • Systemic events (fever, vomiting, headache, fatigue, new or worsening muscle pain, and new or worsening joint pain), as self-reported on e-diaries for up to 7 days following each dose of investigational product. • Nonserious AEs from the signing of the informed consent document (ICD) to 1 month after receipt of the third dose of investigational product. • SAEs from the signing of the ICD to 6 months after receipt of third dose of investigational product.
Evaluation Order	**Secondary Objective(s):**	**Secondary Endpoint(s):**
	3-Dose Family	
1.	• To evaluate the efficacy of Pfizer's *C difficile* vaccine in reducing: • The incidence of all CDI cases (see case definition 1 and case definition 2).	• CDI incidence per 1000 person-years of follow-up, assessed after receipt of the third dose of investigational product onwards.

2.	• To evaluate the efficacy of Pfizer's *C difficile* vaccine in reducing the severity of CDI, defined by:
	  • The duration of CDI episodes.
	  • The requirement to seek medical attention.
		• Mean time to resolution of diarrhea in first primary episodes of CDI (case definition 1).
		• Proportion of subjects experiencing a first primary episode of CDI (case definition 1) who have a non–protocol-related medically attended visit during the CDI episode.
		(Each analysis will be performed only if the preceding one was successful).

3.	• To evaluate the efficacy of Pfizer's *C difficile* vaccine in reducing the incidence of recurrent CDI (see case definition 2).	• CDI incidence per 1000 person-years of follow-up, assessed after receipt of the third dose of investigational product onwards.
	At-Least-2-Dose/Only-2-Dose Family	
1.[b]	• To evaluate the efficacy of Pfizer's *C difficile* vaccine in reducing: • The incidence of all CDI cases (see case definition 1 and case definition 2).	• CDI incidence per 1000 person-years of follow-up, assessed after receipt of the second dose of investigational product onwards.
2.[b]	• To evaluate the efficacy of Pfizer's *C difficile* vaccine in reducing the incidence of recurrent CDI (see case definition 2).	• CDI incidence per 1000 person-years of follow-up, assessed after receipt of the second dose of investigational product onwards.

3.[c]	• In subjects who receive only 2 doses of vaccine, to evaluate the efficacy of Pfizer's *C difficile* vaccine in reducing:
	  • The incidence of a first primary episode of CDI (see case definition 1).
	  • The incidence of recurrent CDI (see case definition 2).
		• CDI incidence per 1000 person-years of follow-up.
		(Each analysis will be performed only if the preceding one was successful).

CCI [redacted]		[redacted]
CCI [redacted]		CCI [redacted]

a. Each subject may contribute only once to the primary endpoint.
b. At-least-2-dose family.
c. Only-2-dose family.

Immunogenicity samples will also be collected during the study. All subjects with a first primary episode of CDI (meeting case definition 1, Section 2.1.1) will be matched with 1 or more control subjects; a separate analysis may be conducted on these case-control data to ascertain if it is possible to determine an immunological correlate of protection.

2.1. CDI Case Definitions

For all definitions, an episode will be considered to have resolved once there have been at least 2 days without passage of 3 or more unformed stools (Bristol stool chart types 5-7) and there is no further need for antibiotic treatment for CDI.

2.1.1. Definition 1 – Primary Episode

A primary episode of CDI (ie, no previous CDI onset in the prior 8 weeks) is defined as either:

- Presence of diarrhea, defined as passage of 3 or more unformed stools (Bristol stool chart types 5-7) in 24 or fewer consecutive hours; and
- Stool sample that is positive for the toxin B gene (by PCR) and positive for toxin A and/or toxin B, as measured in the central laboratory.

or:

- Pseudomembranous colitis diagnosed at colonoscopy, at surgery, or histopathologically; and corresponding stool sample that is positive for the toxin B gene (via PCR) as measured in the central laboratory.

2.1.2. Definition 2 – Recurrent Episode

An episode of CDI that occurs 8 weeks or less after the onset of a previous CDI episode (provided the symptoms of the previous episode had resolved), defined as either:

- Presence of diarrhea, defined as passage of 3 or more unformed stools (Bristol stool chart types 5-7) in 24 or fewer consecutive hours; and
- Stool sample that is positive for the toxin B gene (by PCR) and positive for toxin A and/or toxin B, as measured in the central laboratory.

or:

- Pseudomembranous colitis diagnosed at colonoscopy, at surgery, or histopathologically; and corresponding stool sample that is positive for the toxin B gene (by PCR) as measured in the central laboratory.

CCI [redacted]

3. STUDY DESIGN

This is a Phase 3, placebo-controlled, randomized, observer-blinded study to evaluate the efficacy, safety, and tolerability of AlOH-containing *C difficile* vaccine (200 µg total toxoid) administered as a 3-dose regimen at Months 0, 1, and 6 in adults 50 years of age and above.

Subjects will be randomly assigned in parallel in a 1:1 ratio to receive *C difficile* vaccine (200 µg total toxoid) or placebo (saline).

This is an event-driven study with a total target of 66 first primary CDI cases (meeting case definition 1 in the per-protocol-3 population, Section 2.1.1 and Section 9.2.1) to be accrued for the study; the total enrollment number may vary depending on the incidence rate of the primary endpoint, true underlying VE, and potential early stop for efficacy or futility (see Section 9.2).

3.1. Approximate Number of Subjects

It is anticipated that up to approximately 17,476 adults, 50 years of age and above, may be enrolled globally to accumulate 66 first primary CDI cases (meeting case definition 1, Section 2.1.1). As this is an event-driven and group-sequential (GS) study, the final enrollment number may vary depending on the incidence rate of the primary endpoint, true underlying VE, and potential early stop for efficacy or futility. The number of subjects enrolled in each country and at each site will vary based on enrollment capabilities.

> **Update as Part of Amendment 2 (January 2019):**
> Based on the ongoing blinded review of pooled primary CDI events, it is planned to enroll approximately 1700 additional subjects, giving a total of approximately 17,476 subjects (see Section 9.1).

To achieve a broad representation of age groups among those 50 years and older, and achieve an adequate rate of CDI, the numbers of subjects intended to be enrolled in each of 4 age categories is shown in Table 1.

Table 1. Intended Numbers of Subjects Included in the Study, by Age

Age Category	Minimum Number for Inclusion	Maximum Number for Inclusion
50-59 years	800 subjects	1600 subjects

Table 1. Intended Numbers of Subjects Included in the Study, by Age

Age Category	Minimum Number for Inclusion	Maximum Number for Inclusion
60-69 years	1600 subjects	Not limited
70-79 years	1600 subjects	Not limited
≥80 years	400 subjects	Not limited

On monitoring the pooled primary CDI event rates in different age groups, inclusion criteria subgroups, Charlson Comorbidity Index[61] (CCI) subgroups, and other baseline factors, once the specified minimum numbers in each age group have been achieved, if a substantially higher pooled CDI event rate is observed in some subgroups, the intention may be to stop enrollment in lower-risk subgroups and complete enrollment with the higher-risk subgroups thereafter. This is mainly to help to achieve the targeted total number of first primary cases in a timely manner.

Subjects withdrawn from the study after randomization will not be replaced, regardless of the reason for withdrawal.

3.2. Approximate Duration of the Study

As this study is event-driven, the study duration will be dependent on CDI event rate. The study may be terminated earlier for efficacy or futility as described in Section 9.5.

Given that this is an event-driven study, the end of trial is defined as database lock as described in Section 13.

3.3. Approximate Duration of Subject Participation

Subjects will be followed from the time they sign the ICD until sufficient cases have been accrued to declare efficacy or futility.

4. SUBJECT ELIGIBILITY CRITERIA

This study can fulfill its objectives only if appropriate subjects are enrolled. The following eligibility criteria are designed to select subjects for whom participation in the study is considered appropriate. All relevant medical and nonmedical conditions should be taken into consideration when deciding whether a particular subject is suitable for this protocol.

Subject eligibility should be reviewed and documented by an appropriate member of the investigator's study team before subjects are included in the study.

4.1. Inclusion Criteria

Subjects must meet all of the following inclusion criteria to be eligible for enrollment into the study:

1. Evidence of a personally signed and dated ICD indicating that the subject has been informed of all pertinent aspects of the study.

2. Willing and able to comply with scheduled visits, vaccination plan, and other study procedures.

3. 50 Years or older at enrollment.

4. Subjects with an increased risk of future contact with healthcare systems by virtue of:

   - At least 1 inpatient hospitalization ≥2 nights' duration in the previous 12 months; or

   - At least 2 emergency room visits in the previous 12 months; or

   - At least 10 outpatient visits (primary and/or secondary care visits; defined as an in-person visit to the office/clinic of a prescribing healthcare provider for the purposes of the diagnosis, treatment, or ongoing management of a medical condition, excluding pharmacy and mental health visits) in the previous 12 months; or

   - Residence in a skilled nursing facility (a residential institution that provides professional nursing care and rehabilitation services, usually following discharge from the hospital); or

   - Residence in a nursing home (a residential institution that provides assistance with activities of daily living); or

   - Inpatient hospitalization ≥2 nights' duration scheduled ≥37 days after randomization.

   Or subjects who have received systemic (ie, oral or injected) antibiotics for a minimum of 48 hours at any time in the previous 12 weeks.

5. Ability to be contacted by telephone during study participation.

6. Negative urine pregnancy test for female subjects of childbearing potential.

Female subjects of nonchildbearing potential must meet at least 1 of the following criteria:

a. Achieved postmenopausal status, defined as follows: cessation of regular menses for at least 12 consecutive months with no alternative pathological or physiological cause; status may be confirmed with a serum follicle-stimulating hormone (FSH) level confirming the postmenopausal state;

b. Have undergone a documented hysterectomy and/or bilateral oophorectomy;

c. Have medically confirmed ovarian failure.

All other female subjects (including female subjects with tubal ligations) are considered to be of childbearing potential.

4.2. Exclusion Criteria

Subjects with any of the following characteristics/conditions will not be included in the study:

1. Investigator site staff members directly involved in the conduct of the study and their family members, site staff members otherwise supervised by the investigator, or subjects who are Pfizer employees, including their family members, directly involved in the conduct of the study.

2. Participation in other studies involving investigational drug(s)/vaccine(s) within 28 days prior to study entry until Visit 5 (1 month after the third vaccination).

3. Previous administration of an investigational *C difficile* vaccine or *C difficile* mAb therapy.

4. Prior episode of CDI, confirmed by either laboratory test or diagnosis of pseudomembranous colitis at colonoscopy, at surgery, or histopathologically.

5. Receipt of blood products or immunoglobulins within 6 months before enrollment.

6. Subjects who may be unable to respond to vaccination because of:

   - Metastatic malignancy; or

   - End-stage renal disease (glomerular filtration rate <15 mL/min/1.73 m^2 or on dialysis); or

   - Any serious medical disorder that in the investigator's opinion is likely to be fatal within the next 12 months; or

   - Congenital or acquired immunodeficiency; or

- Receipt of systemic corticosteroids (≥20 mg/day of prednisone or equivalent) for ≥14 days within 28 days of enrollment; or

- Receipt of chronic systemic treatment with other known immunosuppressant medications, or radiotherapy, within 6 months of enrollment.

7. Known infection with human immunodeficiency virus (HIV).

8. Any bleeding disorder or anticoagulant therapy that would contraindicate intramuscular injection.

9. Any contraindication to vaccination or vaccine components, including previous anaphylactic reaction to any vaccine or vaccine-related components.

10. Prior small- or large-bowel resection (does not include appendectomy).

11. Any condition or treatment resulting in frequent diarrhea (≥3 loose stools per day more than once per month), as reported by the subject.

12. Other acute or chronic medical or psychiatric condition including recent (within the past year) or active suicidal ideation or behavioral or laboratory abnormality that may increase the risk associated with study participation or investigational product administration or may interfere with the interpretation of study results and, in the judgment of the investigator, would make the subject inappropriate for entry into this study.

13. Pregnant female subjects; breastfeeding female subjects; fertile male subjects and female subjects of childbearing potential who are, in the opinion of the investigator, sexually active and at risk for pregnancy with their partner(s) and are unwilling or unable to use 2 highly effective methods of contraception as outlined in this protocol from the signing of the informed consent until at least 28 days after the last dose of investigational product.

4.3. Criteria for Temporarily Delaying Vaccine Administration

The following conditions are temporary or self-limiting and a subject may be vaccinated once the condition(s) has/have resolved and no other exclusion criteria are met:

1. Current febrile illness (oral temperature of ≥100.4°F [38.0°C]) or other acute illness (including diarrhea of ≥3 unformed stools within 24 hours) within 48 hours prior to investigational product administration.

2. Subject has received nonadjuvanted seasonal or pandemic influenza vaccine within the previous 14 days or adjuvanted seasonal influenza vaccine or any noninfluenza nonstudy vaccine within the previous 28 days before investigational product administration.

3. If systemic corticosteroids have been administered short term (<14 days) for treatment of an acute illness, investigational product administration should be delayed until systemic corticosteroid use has been discontinued for at least 28 days. Inhaled, intra-articular/intrabursal, or topical corticosteroids are permitted.

If a subject meets any delay criteria for vaccination, all study procedures, including blood sample collection relating to that visit, should be delayed until the day of vaccination.

4.4. Lifestyle Requirements

4.4.1. Contraception

All fertile male subjects and female subjects who are of childbearing potential who are, in the opinion of the investigator, sexually active and at risk for pregnancy with their partner(s) must agree to use 2 highly effective methods of contraception consistently and correctly for the duration of the vaccination period and for at least 28 days after the last dose of investigational product. The investigator or his or her designee, in consultation with the subject, will confirm that the subject has selected 2 appropriate methods of contraception for the individual subject and his or her partner from the permitted list of contraception methods (see below) and will confirm that the subject has been instructed in its consistent and correct use. At time points indicated in the schedule of activities, the investigator or designee will inform the subject of the need to use 2 highly effective methods of contraception consistently and correctly and document the conversation and the subject's affirmation in the subject's chart (subjects need to affirm their consistent and correct use of at least 2 of the selected methods of contraception). In addition, the investigator or designee will instruct the subject to call immediately if 1 or both of the selected contraception methods is discontinued or if pregnancy is known or suspected in the subject or the partner.

Highly effective methods of contraception are those that, alone or in combination, result in a failure rate of less than 1% per year when used consistently and correctly (ie, perfect use) and include the following:

1. Established use of hormonal methods of contraception associated with inhibition of ovulation (eg, oral, inserted, injected, implanted, transdermal), provided the subject or male subject's female partner plans to remain on the same treatment throughout the entire study and has been using that hormonal contraceptive for an adequate period of time to ensure effectiveness.

2. Correctly placed copper-containing intrauterine device (IUD).

3. Male condom or female condom used WITH a separate spermicide product (ie, foam, gel, film, cream, or suppository). For countries where spermicide is not available or condom plus spermicide is not accepted as highly effective contraception, this option is not appropriate.

4. Male sterilization with absence of sperm in the postvasectomy ejaculate.

5. Bilateral tubal ligation/bilateral salpingectomy or bilateral tubal occlusive procedure (provided that occlusion has been confirmed in accordance with the device's label).

NOTE: Sexual abstinence, defined as completely and persistently refraining from all heterosexual intercourse (including during the entire period of risk associated with the study treatments) may obviate the need for contraception ONLY if this is the preferred and usual lifestyle of the subject.

4.5. Sponsor's Qualified Medical Personnel

The contact information for the sponsor's appropriately qualified medical personnel for the study is documented in the study contact list located in the study reference manual (SRM).

To facilitate access to appropriately qualified medical personnel on study-related medical questions or problems, subjects are provided with a contact card. The contact card contains, at a minimum, protocol and investigational product identifiers, subject study numbers, contact information for the investigator site, and contact details for a contact center in the event that the investigator site staff cannot be reached to provide advice on a medical question or problem originating from another healthcare professional not involved in the subject's participation in the study. The contact number can also be used by investigator staff if they are seeking advice on medical questions or problems; however, it should be used only in the event that the established communication pathways between the investigator site and the study team are not available. It is therefore intended to augment, but not replace, the established communication pathways between the investigator site and the study team for advice on medical questions or problems that may arise during the study. The contact number is not intended for use by the subject directly, and if a subject calls that number, he or she will be directed back to the investigator site.

5. INVESTIGATIONAL PRODUCTS

For the purposes of this study, and per International Conference on Harmonisation (ICH) guidelines, investigational product is defined as a pharmaceutical form of an active ingredient or placebo being tested or used as a reference/comparator in a clinical trial, including a product with a marketing authorization when used or assembled (formulated or packaged) in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use (ICH E6 1.33).

For this study, the investigational products are *C difficile* vaccine and placebo (saline). Since the appearance of these investigational products is not identical, the study is observer-blinded.

5.1. Allocation to Investigational Product

Allocation of subjects to vaccine groups will proceed through the use of an interactive response technology (IRT) system (interactive Web-based response [IWR]). Study personnel (either blinded or unblinded) will be required to enter or select information including but not limited to the user's identification (ID) and password, the protocol number, and the subject number. They will then be provided with a randomization number, vaccine assignment, and

dispensable unit (DU) or container number. The IRT system will provide a confirmation report containing the subject number, randomization number, and DU or container number assigned. The confirmation report must be retained by the unblinded dispenser in the unblinded site files.

The study-specific IRT reference manual will provide the contact information and further details on the use of the IRT system.

5.2. Blinding of Site Personnel

In this observer-blinded study, the study staff dispensing and administering the vaccine will be unblinded, but all other site study personnel, including the principal investigator and the subject, will be blinded.

The principal investigator will assign the responsibility of the unblinded dispenser/administrator to persons who will not participate in the evaluation of any study subject. More than 1 unblinded dispenser/administrator will be assigned per site. A member of the study site staff or clinic pharmacy should fulfill this role. Contact between the unblinded dispenser and study subjects should be kept to a minimum. The investigator, study coordinator, and any site staff other than the unblinded dispenser/administrator must not be allowed to know the investigational product assigned to any study subject and must not be allowed to see the investigational product container contents.

5.3. Blinding of the Sponsor

Those study team members who are involved in ensuring that protocol requirements for investigational product handling, allocation, and administration are fulfilled at the site (eg, study manager; clinical research associates [CRAs]) will be unblinded for the duration of the study. All other study team members and all laboratory personnel performing the stool and serology assays will remain blinded to vaccine assigned/received throughout the study.

5.4. Breaking the Blind

The study will be subject and investigator blinded.

At the initiation of the study, the investigator site will be instructed on the method for breaking the blind. The method will be an electronic process. Blinding codes should be broken only in exceptional circumstances when knowledge of the actual vaccine assignment is absolutely essential for further management of the subject. Investigators are encouraged to discuss with a member of the study team if they believe that unblinding is necessary. When the blinding code is broken, the reason must be fully documented and entered on the case report form (CRF).

5.5. Subject Compliance

All doses of investigational product will be administered by the appropriately designated study staff at the investigator site.

5.6. Vaccine Supplies

5.6.1. Formulation and Packaging

5.6.1.1. *Clostridium difficile* Vaccine

The investigational *C difficile* vaccine is toxoid based. C *difficile* toxin A and toxin B are inactivated by a combination of genetic mutations and chemical treatments. The vaccine is provided as a sterile lyophilized powder in a dosage strength of 200 µg/dose (total for toxoids A and B). The vaccine will be reconstituted with AlOH diluent immediately before use as instructed in the investigational product (IP) manual. The AlOH diluent is supplied as a 1-mg aluminum/mL (as AlOH) liquid suspension.

Blinded, sealed cartons will contain 1 single-use vial of *C difficile* vaccine lyophilized powder for reconstitution and 1 single-dose syringe of AlOH diluent. The total volume of resuspended vaccine to be injected is 0.5 mL per dose.

The investigational products will be provided by the sponsor to each study site. Investigational product and diluent will be packed and labeled by Pfizer or its designee as investigational product in accordance with current guidelines and applicable local and legal regulatory requirements. For detailed information on the antigenic components of the investigational product, please refer to the IB.

5.6.1.2. Placebo

The placebo will consist of a sterile normal saline solution for injection (0.9% sodium chloride injection, in a 0.5-mL dose) and will be provided by the sponsor to each study site. The placebo will be packaged within cartons labeled by Pfizer or its designee in accordance with current guidelines and applicable local and legal regulatory requirements. Blinded, sealed cartons will contain 1 single-dose syringe of placebo. Refer to the IP manual for details regarding the preparation and dispensing of placebo.

5.6.2. Preparation and Dispensing

See the IP manual for instructions on how to prepare the investigational product for administration. Investigational product should be prepared and dispensed by an appropriately qualified and experienced **unblinded** member of the study staff.

5.6.3. Administration

All injections will be administered in the upper deltoid muscle, preferably of the nondominant arm, by the **unblinded** administrator.

Subjects will receive 1 dose of *C difficile* vaccine/placebo at Visits 1 (Month 0), 2 (Month 1), and 4 (Month 6).

Standard vaccination practices must be observed and vaccine must not be injected into blood vessels. Appropriate medication and other supportive measures for management of an acute hypersensitivity reaction should be available in accordance with local guidelines for standard immunization practices.

Administration of investigational products should be performed by an appropriately qualified, Good Clinical Practice (GCP)-trained, and vaccine-experienced member of the study staff (eg, physician, nurse, physician's assistant, nurse practitioner, pharmacist, or medical assistant) as allowed by local, state, and institutional guidance.

Investigational product administration details will be recorded on the CRF.

5.7. Investigational Product Storage

The investigator or an approved representative, eg, pharmacist, will ensure that all investigational products are stored in a secured area with controlled access under required storage conditions and in accordance with applicable regulatory requirements.

Investigational products should be stored in their original containers and in accordance with the labels.

See the IP manual for storage conditions of the product once reconstituted.

Any storage conditions stated in the SRSD will be superseded by the storage conditions stated on the product label.

The investigational vaccine, AlOH diluent, and placebo will be shipped at +2°C to +8°C to each study site after required regulatory and legal documents have been received by the sponsor. Upon receipt at the study site, the products should be immediately transferred to a +2°C to +8°C temperature-monitored refrigerator for storage.

Site systems must be capable of measuring and documenting (for example, via a log), at a minimum, daily minimum and maximum temperatures for all site storage locations (as applicable, including frozen, refrigerated, and/or room-temperature products). This should be captured from the time of investigational product receipt throughout the study. Even for continuous-monitoring systems, a log or site procedure that ensures active evaluation for excursions should be available. The intent is to ensure that the minimum and maximum temperature is checked each business day to confirm that no excursion occurred since the last evaluation and to provide the site with the capability to store or view the minimum/maximum temperature for all nonworking days upon return to normal operations. The operation of the temperature-monitoring device and storage unit (for example, refrigerator), as applicable, should be regularly inspected to ensure they are maintained in working order.

Any excursions from the product label storage conditions should be reported to Pfizer upon discovery. The site should actively pursue options for returning the product to the storage conditions described in the labeling, as soon as possible. Deviations from the storage requirements, including any actions taken, must be documented and reported to Pfizer.

Once an excursion is identified, the investigational product must be quarantined and not used until Pfizer provides permission to use the investigational product. It will not be considered a protocol deviation if Pfizer approves the use of the investigational product after the temperature excursion. Use of the investigational product prior to Pfizer approval will be

considered a protocol deviation. Specific details regarding information the site should report for each excursion will be provided to the site.

Receipt of materials, door opening and closing, and other routine handling operations where the products are briefly out of the temperature range described in the labeling are not considered excursions.

5.8. Investigational Product Accountability

The investigator site must maintain adequate records documenting the receipt, use, loss, or other disposition of the investigational product supplies. All investigational products will be accounted for using a drug accountability form/record.

5.8.1. Destruction of Investigational Product Supplies

The sponsor or designee will provide guidance on the destruction of unused investigational product (eg, at the site). If destruction is authorized to take place at the investigator site, the investigator must ensure that the materials are destroyed in compliance with applicable environmental regulations, institutional policy, and any special instructions provided by Pfizer, and all destruction must be adequately documented.

5.9. Concomitant Medication(s)

5.9.1. Recording Concomitant Vaccinations and Medications

The following concomitant medications and vaccinations will be recorded in the CRF:

- All vaccinations received from 28 days prior to study enrollment until Visit 5 (Month 7 [1 month after the third vaccination]).
- At the time of a suspected CDI case, start date, name of medication, dose, unit, route, and frequency will be recorded for the following:
    - Antibiotic use within the 12 weeks prior to the onset of the suspected CDI case.
    - Antibiotic use for the treatment of the suspected CDI case.
    - Known immunosuppressant medication, or radiotherapy use, within the 6 months prior to the onset of the suspected CDI case.
    - Systemic corticosteroid use within the 28 days prior to the onset of the suspected CDI case.
    - Proton-pump inhibitor use within the 12 weeks prior to the onset of the suspected CDI case.

5.9.2. Prohibited Concomitant Vaccinations and Medications

Receipt of the following prohibited vaccines and medications during the time periods listed below may exclude a subject from the per-protocol analysis, and may require vaccinations to be discontinued in that subject; however, it is anticipated that the subject would not be withdrawn from the study (see Section 6.2).

Unless considered medically necessary, no vaccines other than investigational product should be administered within 28 days before and 28 days after each study vaccination (administered at Visits 1, 2, and 4). One exception to this is that nonadjuvanted seasonal influenza vaccine and pandemic influenza vaccine can be given at least 14 days after, or at least 14 days prior to, the administration of investigational product.

Receipt of chronic systemic treatment with other known immunosuppressant medications, or radiotherapy, within 6 months before enrollment through conclusion of the study.

Receipt of systemic corticosteroids (≥20 mg/day of prednisone or equivalent) for ≥14 days is prohibited from 28 days prior to enrollment to Visit 5 (Month 7).

Receipt of blood products or immunoglobulins within 6 months before enrollment through conclusion of the study.

Receipt of any other (nonstudy) investigational *C difficile* vaccine or *C difficile* mAb therapy at any time prior to or during study participation is prohibited.

Prophylactic antipyretics and other pain medication to prevent symptoms associated with investigational product administration are not permitted. However, if a subject is taking a medication for another condition, even if it may have antipyretic or pain-relieving properties, it should not be withheld prior to study vaccination.

5.9.3. Permitted Concomitant Medications

The use of antipyretics and other pain medication to treat symptoms associated with investigational product administration or ongoing conditions is permitted.

Medication other than that described as prohibited in Section 5.9.2 required for treatment of preexisting stable conditions is permitted.

Inhaled, topical, or localized injections of corticosteroids (eg, intra-articular or intrabursal administration) are permitted.

6. STUDY PROCEDURES

6.1. Study Period

Throughout the study period, if a subject has had no study-related activity for at most 1 month, the subject will receive an electronic reminder to confirm that he or she is still active in the study and to remind the subject to record when he or she experiences an unformed stool and to collect a sample when required (see Section 6.1.9 and Section 6.4).

If the subject is unresponsive to the electronic reminder, the site staff should contact him or her by telephone. Site staff will be able to record that a subject is still active in the electronic diary (e-diary) vendor portal. In addition, all subjects will receive a reminder every 2 weeks to collect a stool sample should a diarrheal episode occur (3 or more diarrheal episodes, Bristol type 5-7, within 24 hours).

Deaths will be recorded for the entire study duration, as follows:

a. Occurring within 30 days of a diarrheal episode: death should be recorded on the relevant CRF page related to the diarrheal episode (see Section 6.1.9). In addition, dependent on the timing within the study, b or c below should also be followed.

b. Occurring between provision of informed consent and Visit 6: the underlying cause should be reported as an SAE (see Section 8.2.3) with death as the outcome.

c. Occurring after Visit 6 and before the end of the study: the date and cause of death should be recorded on the relevant CRF page. For reporting requirements outside of the active collection period, see Section 8.1.4.1.

6.1.1. Visit 1: Month 0 – Vaccination 1

It is anticipated that the procedures below will be conducted in a stepwise manner; ensure that procedures listed prior to administration of the vaccine are conducted prior to vaccination.

- Obtain written informed consent prior to performing any protocol required procedures.
- Record the subject's demography (including date of birth, sex, race, and ethnicity).
- Conduct a clinical assessment, including review of medical history, to verify that all inclusion criteria, none of the exclusion criteria, and none of the temporary delay criteria are met (Section 4). Record medical history of significance, including the presence of chronic medical conditions. If the clinical assessment indicates that a physical examination is necessary to comprehensively evaluate the subject, perform a physical examination and record the findings.
- Record nonstudy vaccines as described in Section 5.9.1.
- Measure and record the subject's weight and height.
- Measure and record the subject's oral temperature.
- For female subjects of childbearing potential, perform a urine pregnancy test and ensure the result is negative prior to vaccination (Section 7.5).
- Discuss contraceptive use.

- Ensure that all inclusion criteria, none of the exclusion criteria, and none of the temporary delay criteria are met (Section 4).

- An appropriate site staff member (may be blinded or unblinded) will use the IRT system to obtain the subject's randomization number and investigational product blinded carton number.

- Collect a blood sample of approximately 20 mL for immunogenicity testing (Section 7.3).

- Unblinded site staff will prepare and administer investigational product. Investigational product will be administered by intramuscular injection into the upper deltoid muscle, preferably of the nondominant arm. The time of vaccination will be recorded. Refer to the IP manual under separate cover for further instruction on this process.

- The unblinded vaccine dispenser/administrator updates the investigational product accountability records.

- Blinded study staff will observe the subject for at least 30 minutes after investigational product administration for any acute reactions. Any AEs noted during the observation period should be recorded in the CRF.

- Record and report AEs and SAEs (relative to the time of vaccination) as described in Section 8 and the schedule of activities.

- Review the stool sample collection and shipping instructions with the subject and ensure the subject acknowledges understanding, including the requirement to contact the courier for sample collection (see Section 6.3).

- Issue the stool collection kit and shipping box and provide instructions on their use (see Section 6.3).

- Explain the communication technologies available for the study (which also allow for the completion of the e-diary), and assist the subject in downloading the study application (app) onto the subject's own device or issue a provisioned device if required (see Section 6.4).

- Issue a measuring device for measurement of local reactions, and a digital thermometer for recording daily temperatures. Provide instructions on their use and recording.

- Explain the e-diary platform to collect vaccine reactogenicity data and provide instructions on its completion. Ask the subject to complete the e-diary from Day 1 to Day 7, with Day 1 being the day of vaccination.

- Ask the subject to contact the site staff or investigator immediately if he or she experiences 3 or more unformed stools (Bristol stool chart types 5-7) in any 24-hour period.

- Ask the subject to contact the site staff or investigator immediately if any significant illness or hospitalization occurs.
- Schedule an appointment for the subject to return for the next study visit.
- Complete the source documents.
- Complete the CRF.

6.1.2. Visit 2: Month 1 – Vaccination 2 (28 to 42 Days After Visit 1)

It is anticipated that the procedures below will be conducted in a stepwise manner; ensure that procedures listed prior to administration of the vaccine are conducted prior to vaccination.

- Review the subject's e-diary data and follow up on any ongoing local reactions or systemic events.
- Ensure that the subject continues to be eligible for the study and does not meet any of the withdrawal criteria as described in Section 6.2.
- Discuss with the subject to determine whether any major protocol violations have occurred, as described in Section 6.2.1, and record in source documents.
- Based on clinical evaluation, determine whether any AEs or SAEs have occurred since the last study visit. Record and report findings as described in Section 8 and the schedule of activities. Review AEs that were ongoing from the previous visit and record their stop dates or confirm if they are still continuing.
- Record nonstudy vaccines as described in Section 5.9.1.
- Measure and record the subject's oral temperature.
- For female subjects of childbearing potential, perform a urine pregnancy test and ensure the result is negative prior to vaccination (Section 7.5).
- Discuss contraceptive use.
- Ensure that none of the temporary delay criteria are met (Section 4.3).
- An appropriate site staff member (may be blinded or unblinded) will use the IRT system to obtain the subject's investigational product blinded carton number.
- Unblinded site staff will prepare and administer investigational product. Investigational product will be administered by intramuscular injection into the upper deltoid muscle, preferably of the nondominant arm. The time of vaccination will be recorded. Refer to the IP manual under separate cover for further instruction on this process.

- The unblinded vaccine dispenser/administrator updates the investigational product accountability records.

- Blinded study staff will observe the subject for at least 30 minutes after investigational product administration for any acute reactions. Any AEs noted during the observation period should be recorded in the CRF.

- Record and report AEs and SAEs (relative to the time of vaccination) as described in Section 8 and the schedule of activities.

- Confirm that the subject understands the stool sample collection and shipping requirements, including the mechanism to contact the courier for sample collection (see Section 6.3).

- Ensure the subject has an available stool collection kit and shipping box and provide instructions on their use, as required (see Section 6.3).

- Ensure the subject is comfortable with his or her chosen communication technology (see Section 6.4).

- Ensure the subject has an available measuring device for measurement of local reactions, and a digital thermometer for recording daily temperatures. If required, provide instructions on their use.

- Confirm that the subject understands the reactogenicity reporting requirements. Remind the subject to complete the e-diary from Day 1 to Day 7, with Day 1 being the day of vaccination.

- Ask the subject to contact the site staff or investigator immediately if he or she experiences 3 or more unformed stools in any 24-hour period.

- Ask the subject to contact the site staff or investigator immediately if any significant illness or hospitalization occurs.

- Schedule an appointment for the subject to return for the next study visit.

- Complete the source documents.

- Complete the CRF.

6.1.3. Visit 3: Month 2 (28 to 42 Days After Visit 2)

- Review the subject's e-diary data and follow up on any ongoing local reactions or systemic events.

- Ensure that the subject continues to be eligible for the study and does not meet any of the withdrawal criteria as described in Section 6.2.

- Discuss with the subject to determine whether any major protocol violations have occurred, as described in Section 6.2.1, and record in source documents.

- Based on clinical evaluation, determine whether any AEs or SAEs have occurred since the last study visit. Record and report findings as described in Section 8 and the schedule of activities. Review AEs that were ongoing from the previous visit and record their stop dates or confirm if they are still continuing.

- Record nonstudy vaccines as described in Section 5.9.1.

- Collect a blood sample of approximately 20 mL for immunogenicity testing (Section 7.3).

- Discuss contraceptive use.

- Confirm that the subject understands the stool sample collection and shipping requirements, including the mechanism to contact the courier for sample collection (see Section 6.3).

- Ensure the subject has an available stool collection kit and shipping box and provide instructions on their use, as required (see Section 6.3).

- Ensure the subject is comfortable with his or her chosen communication technology (see Section 6.4).

- Ask the subject to contact the site staff or investigator immediately if he or she experiences 3 or more unformed stools in any 24-hour period.

- Ask the subject to contact the site staff or investigator immediately if any significant illness or hospitalization occurs.

- Schedule an appointment for the subject to return for the next study visit.

- Complete the source documents.

- Complete the CRF.

6.1.4. Visit 4: Month 6 – Vaccination 3 (140 to 168 Days After Visit 2)

It is anticipated that the procedures below will be conducted in a stepwise manner; ensure that procedures listed prior to administration of the vaccine are conducted prior to vaccination.

- Review the subject's e-diary data and follow up on any ongoing local reactions or systemic events.

- Ensure that the subject continues to be eligible for the study and does not meet any of the withdrawal criteria as described in Section 6.2.

- Discuss with the subject to determine whether any major protocol violations have occurred, as described in Section 6.2.1, and record in source documents.

- Based on clinical evaluation, determine whether any AEs or SAEs have occurred since the last study visit. Record and report findings as described in Section 8 and the schedule of activities. Review AEs that were ongoing from the previous visit and record their stop dates or confirm if they are still continuing.

- Record nonstudy vaccines and as described in Section 5.9.1.

- Measure and record the subject's oral temperature.

- For female subjects of childbearing potential, perform a urine pregnancy test and ensure the result is negative prior to vaccination (Section 7.5).

- Discuss contraceptive use.

- Ensure that none of the temporary delay criteria are met (Section 4.3).

- An appropriate site staff member (may be blinded or unblinded) will use the IRT system to obtain the subject's investigational product blinded carton number.

- Unblinded site staff will prepare and administer investigational product. Investigational product will be administered by intramuscular injection into the upper deltoid muscle, preferably of the nondominant arm. The time of vaccination will be recorded. Refer to the IP manual under separate cover for further instruction on this process.

- The unblinded vaccine dispenser/administrator updates the investigational product accountability records.

- Blinded study staff will observe the subject for at least 30 minutes after investigational product administration for any acute reactions. Any AEs noted during the observation period should be recorded in the CRF.

- Record and report AEs and SAEs (relative to the time of vaccination) as described in Section 8 and the schedule of activities.

- Confirm that the subject understands the stool sample collection and shipping requirements, including the mechanism to contact the courier for sample collection (see Section 6.3).

- Ensure the subject has an available stool collection kit and shipping box and provide instructions on their use, as required (see Section 6.3).

- Ensure the subject has an available measuring device for measurement of local reactions, and a digital thermometer for recording daily temperatures. If required, provide instructions on their use.

- Ensure the subject is comfortable with his or her chosen communication technology (see Section 6.4).
- Remind the subject to complete the e-diary from Day 1 to Day 7, with Day 1 being the day of vaccination.
- Ask the subject to contact the site staff or investigator immediately if he or she experiences 3 or more unformed stools in any 24-hour period.
- Ask the subject to contact the site staff or investigator immediately if any significant illness or hospitalization occurs.
- Schedule an appointment for the subject to return for the next study visit.
- Complete the source documents.
- Complete the CRF.

6.1.5. Visit 5: Month 7 (28 to 42 Days After Visit 4)

- Review the subject's e-diary data and follow up on any ongoing local reactions or systemic events.
- Discuss with the subject to determine whether any major protocol violations have occurred, as described in Section 6.2.1, and record in source documents.
- Based on clinical evaluation, determine whether any AEs or SAEs have occurred since the last study visit. Record and report findings as described in Section 8 and the schedule of activities. Review AEs that were ongoing from the previous visit and record their stop dates or confirm if they are still continuing.
- Record nonstudy vaccines and as described in Section 5.9.1.
- Collect a blood sample of approximately 20 mL for immunogenicity testing (Section 7.3).
- Confirm that the subject understands the stool sample collection and shipping requirements, including the mechanism to contact the courier for sample collection (see Section 6.3).
- Ensure the subject has an available stool collection kit and shipping box and provide instructions on their use, as required (see Section 6.3).
- Ensure the subject is comfortable with his or her chosen communication technology (see Section 6.4).
- Ask the subject to contact the site staff or investigator immediately if he or she experiences 3 or more unformed stools in any 24-hour period.

- Ask the subject to contact the site staff or investigator immediately if any significant illness or hospitalization occurs.
- Schedule an appointment for the subject to return for the next study visit.
- Complete the source documents.
- Complete the CRF.

6.1.6. Visit 6: Month 12 (165 to 195 Days After Visit 4)

- Visit 6 is intended to be conducted by telephone but, if desired, may be conducted in person.
- Discuss with the subject to determine whether any major protocol violations have occurred, as described in Section 6.2.1, and record in source documents.
- Following the provided script (found in the SRM), contact the subject by telephone to determine whether any SAEs have occurred since the last study visit. Record and report findings as described in Section 8 and the schedule of activities. Review AEs that were ongoing from the previous visit and record their stop dates or confirm if they are still continuing.
- Confirm that the subject understands the stool sample collection and shipping requirements, including the mechanism to contact the courier for sample collection (see Section 6.3).
- Ensure the subject has an available stool collection kit and shipping box and provide instructions on their use, as required (see Section 6.3).
- Ensure the subject is comfortable with his or her chosen communication technology (see Section 6.4).
- Ask the subject to contact the site staff or investigator immediately if he or she experiences 3 or more unformed stools in any 24-hour period.
- Complete the source documents.
- Complete the CRF.

6.1.7. Annual Retention Visits (Every 280 to 392 Days After Visit 5)

As the study is event driven, it is imperative that subjects remain engaged and comfortable with the study requirements. As the study duration increases, in-person retention visits will be used to facilitate this intent.

An annual retention visit should be arranged at the earliest opportunity for subjects already enrolled in the study, following approval of Amendment 3, and then every 280 to 392 days thereafter.

At the time of a retention visit:

- At the first annual retention visit or potential CDI visit, whichever comes first following approval of Amendment 3, obtain written informed consent to the amendments in the protocol.
- Discuss with the subject to determine whether any major protocol violations have occurred, as described in Section 6.2.1, and record in source documents.
- Confirm that the subject understands the stool sample collection and shipping requirements, including the mechanism to contact the courier for sample collection (see Section 6.3).
- Ensure the subject has an available stool collection kit and shipping box and provide instructions on their use, as required (see Section 6.3).
- Ensure the subject is comfortable with his or her chosen communication technology (see Section 6.4).
- Ask the subject to contact the site staff or investigator immediately if he or she experiences 3 or more unformed stools in any 24-hour period.
- Complete the source documents.
- Complete the CRF.

6.1.8. Visit 7: End of Study

- Discuss with the subject to determine whether any major protocol violations have occurred, as described in Section 6.2.1, and record in source documents.
- Telephone the subject and advise him or her that the study has ended.
- Instruct the subject how to return the study materials, including a provisioned device, if applicable.

6.1.9. At the Time of a Potential CDI Case (Follow-up Related to Each Diarrheal Episode)

When the subject experiences 3 or more unformed stools (Bristol stool chart types 5-7) in a 24-hour period, having collected the third or subsequent stool for provision to the central laboratory, he or she is instructed to contact the site. This contact will trigger the following procedures that will include at least 1 in-person visit (eg, at the subject's home, at the study

site, or in a medically attended setting) and may necessitate additional telephone follow-up with the subject. Information will be recorded in the CRF:

- Collect a blood sample of approximately 20 mL (a minimum of 10 mL and up to 20 mL) for immunogenicity testing (Section 7.3). Optimally, this should be collected within 48 hours of the third unformed stool (Bristol stool chart types 5-7).
- Record any AEs occurring up to 48 hours after the blood draw.
- Collect the following information, including but not limited to:
    - Start and stop dates of the diarrheal episode.
    - Total number of unformed stools (Bristol stool chart types 5-7).
    - Type and result of any microbiological test performed locally for detection of *C difficile* and its toxins.
    - Concomitant medications received (see Section 5.9.1).
    - Number and nature of any non–protocol-related medically attended visits up to 1 month after the resolution of the episode.
    - Any new diagnosis (since enrollment) of metastatic malignancy, end-stage renal disease (glomerular filtration rate <15 mL/min/1.73 m^2 or on dialysis), or infection with HIV.
    - If hospitalized: serum creatinine, serum albumin, total leukocyte count, results of microbiological tests performed for detection of stool pathogens other than *C difficile*, nonmicrobiological diagnostic procedures performed (eg, computed tomography scan, endoscopy), length of stay, number of days spent in intensive care, requirement for mechanical ventilation, presence of renal failure, requirement for parenteral nutrition, surgical procedures performed.
- Discuss with the subject to determine whether any major protocol violations have occurred, as described in Section 6.2.1, and record in source documents.

6.1.10. Unscheduled Visit for a Grade 3 or Suspected Grade 4 Reaction

If a Grade 3 local reaction (Section 7.2.1.2), systemic event (Section 7.2.1.3), or fever (Section 7.2.1.4) is reported in the e-diary, a telephone contact should occur to ascertain further details and determine whether a site visit is clinically indicated.

If a suspected Grade 4 local reaction (Section 7.2.1.2), systemic event (Section 7.2.1.3), or fever (Section 7.2.1.4) is reported, a telephone contact or site visit should occur to confirm whether the event meets the criteria for Grade 4.

6.2. Subject Withdrawal

Subject eligibility must be confirmed prior to each vaccination in order to continue in the study.

If a subject no longer meets the eligibility criteria during the vaccination period of the study, discontinuation of further vaccinations should be considered, but the subject may remain in the study and be followed up per the schedule of activities. If a subject no longer meets the eligibility criteria after the vaccination period of the study, and is willing to remain in the study, the subject may do so and will be followed up per the schedule of activities.

Subjects may withdraw from the study at any time at their own request, or they may be withdrawn at any time at the discretion of the investigator or sponsor for safety (see also the Withdrawal From the Study Due to Adverse Events section) or behavioral reasons, or the inability of the subject to comply with the protocol-required schedule of study visits or procedures at a given study site.

If a subject does not return for a scheduled visit, every effort should be made to contact the subject. All attempts to contact the subject and information received during contact attempts must be documented in the subject's medical record. In any circumstance, every effort should be made to document subject outcome, if possible. The investigator should inquire about the reason for withdrawal, request that the subject return for a final visit, if applicable, and follow up with the subject regarding any unresolved AEs.

If the subject withdraws from the study, and also withdraws consent for disclosure of future information, no further evaluations should be performed, and no additional data should be collected. The sponsor may retain and continue to use any data collected before such withdrawal of consent.

6.2.1. Major Protocol Violations

In order to define the per-protocol analysis populations (Section 9.2), it is important to determine which subjects have experienced what may constitute a major protocol violation at any time during the study. At each study contact after Visit 1, site staff should record if any of the following has occurred:

- Subject met exclusion criteria 1, 2, 6, 7, 8, 9, 12, and 13 (Section 4.2).
- Subject in whom vaccination was not appropriately delayed following receipt of short-term (<14 days) systemic corticosteroids for treatment of an acute illness (Section 4.3).
- Subject received chronic systemic treatment with other known immunosuppressant medications, or radiotherapy, through the conclusion of the study (Section 5.9.2).
- Subject received systemic corticosteroids (≥20 mg/day of prednisone or equivalent) for ≥14 days through Visit 5 (Month 7) (Section 5.9.2).

- Receipt of blood products or immunoglobulins through the conclusion of the study (Section 5.9.2).

6.3. Stool Sample Collection

Stool samples (the third or subsequent unformed stool [Bristol stool chart types 5-7] in a 24-hour period) will be collected in either the subject's home or a medical setting (eg, hospital, nursing home). Site staff will review the sample collection and shipping instructions with each subject and ensure that the subject understands the appropriate procedures for collecting and properly packaging stool samples. All subjects will be provided with a stool sample collection kit and shipping box at Visit 1 and with replacement kits as needed throughout the study. For some subjects, assistance may be provided for stool collection from the subject's own caregiver, healthcare assistants, community nurses, site staff, or equivalent, if required.

The process for stool collection and transport to the processing center is detailed in the SRM.

6.4. Subject Communication and Use of Technology

In a study of this duration that is event-driven, it is vital to ensure that communication between the study site and the subject is maintained to ensure that endpoint events are not missed. This study will employ various methods, tailored to the individual subject, to ensure that communication is maintained and study information can be transmitted securely. Using appropriate technology, a communication pathway between the subject, the site, and the stool sample courier will be established. Subjects may be able to utilize their own devices to access this technology, or use a device provided by the sponsor. Traditional methods of telephone communication will also be available. The technology solution may facilitate:

- Contact to the courier for stool sample shipping.
- Contact to the investigator.
- The subject to record individual unformed stools, and alert the subject of a third unformed stool in 24 hours (a diarrheal event).
- An alert in the event a subject is hospitalized.
- Access to study information, including stool collection instructions.
- Visit reminders.
- Reminders to ensure stool sample collection and shipping for diarrheal episodes.
- Messages of thanks and encouragement from the study team.
- A platform for recording local reactions and systemic events (e-diary).

7. ASSESSMENTS

Every effort should be made to ensure that the protocol-required tests and procedures are completed as described. However, it is anticipated that from time to time there may be circumstances outside of the control of the investigator that may make it unfeasible to perform the test. In these cases the investigator will take all steps necessary to ensure the safety and well-being of the subject. When a protocol-required test cannot be performed, the investigator will document the reason for this and any corrective and preventive actions that he or she has taken to ensure that normal processes are adhered to as soon as possible. The study team will be informed of these incidents in a timely manner.

For samples being collected and shipped, detailed collection, processing, storage, and shipment instructions and contact information will be provided to the investigator site prior to initiation of the study.

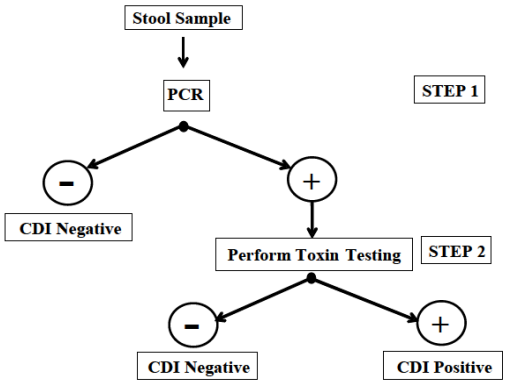
7.1. Efficacy Assessments

Any time during the study period that a subject experiences passage of 3 or more unformed stools (Bristol stool chart types 5-7) within 24 hours, the subject will be asked to collect the third or subsequent stool for provision to the central laboratory. The subject will be provided with a sample collection kit and be instructed on how to package the sample. The sample will be shipped to a processing laboratory. The processing laboratory will prepare aliquots of the stool for later analysis at the central laboratory.

7.1.1. Central Laboratory Testing for *Clostridium difficile*

All diarrheal specimens submitted to the central laboratory will initially be tested by PCR to detect *C difficile* that harbors TcdB. PCR-positive samples will be tested in a second step for presence of free toxins (Figure 1).

Figure 1. Two-Step Testing Algorithm for Determination of *Clostridium difficile* Infection Case Definition



Abbreviations: CDI = *Clostridium difficile* infection; PCR = polymerase chain reaction.

7.1.1.1. Detection of Toxigenic *Clostridium difficile* by PCR

A commercially available, Food and Drug Administration (FDA)-approved and Conformité Européenne (CE)-marked assay will be used for the rapid detection of toxigenic *C difficile* and for the presumptive identification of 027/NAP1/BI strains of toxigenic *C difficile* from the subjects' stool samples. The test is designed to detect the toxin B gene (*tcdB*) sequences associated with toxin-producing *C difficile*. PCR results and the corresponding action taken for the tested samples are outlined in Table 2.

Table 2. Cepheid Xpert *C. difficile*/*Epi* Results

Result	Action
Toxigenic *C difficile* negative; 027 presumptive negative	Sample is reported as *C difficile* negative. No further testing
Toxigenic *C difficile* positive; 027 presumptive negative	Test sample for the presence of free toxins
Toxigenic *C difficile* positive; 027 presumptive positive	Test sample for the presence of free toxins
Invalid	Sample is retested

Note: The Cepheid Xpert *C. difficile/Epi* is a test for detection and differentiation of *Clostridium difficile* and the epidemic 027 strain.

7.1.1.2. Detection of Free Toxins in the Stool Sample

The assay used to detect presence of free toxins will be described in the laboratory manual maintained by the sponsor.

7.1.2. Diagnosis of Pseudomembranous Colitis

The primary CDI case definition can also be met via a definitive demonstration of pseudomembranous colitis and corresponding stool sample that is positive for the toxin B gene (by PCR) as measured in the central laboratory. The diagnosis of pseudomembranous colitis could be made during colonoscopy or surgery or by histopathological examination.

7.2. Safety Assessments

Safety parameters will be assessed as described in the Schedule of Activities, Section 6, Section 8, and below.

A clinical assessment, including medical history, will be performed on all subjects at Visit 1 to establish a baseline. Significant medical history and observations from any physical examination, if performed, will be documented in the CRF.

AEs and SAEs are collected, recorded, and reported as defined in Section 8.

Acute reactions within the first 30 minutes after investigational product administration will be assessed and documented in the AE CRF.

The safety parameters also include e-diary reports of local reactions and systemic events that occur in the 7 days after investigational product administration. These prospectively collected occurrences of local reactions and systemic events are graded as described in Section 7.2.1.

7.2.1. Electronic Diary

Subjects will be required to use an e-diary, based on appropriate technology, and will be asked to monitor and record local reactions and systemic events for 7 days following vaccination. The e-diary allows recording of these assessments only within a fixed time window, thus providing the accurate representation of the subject's experience at that time. Data on local reactions and systemic events reported on the e-diary will be transferred electronically to a third-party vendor, where they will be available for review by investigators and the Pfizer clinicians at all times via an Internet-based portal. E-diary data review is ongoing during subject e-diary data-entry periods (7 days after each vaccination) via an Internet-based portal.

At intervals agreed to by the vendor and Pfizer, these data will be transferred electronically into Pfizer's database for analysis and reporting. These data do not need to be reported by the investigator in the CRF as AEs.

Investigators (or designee) will be required to review the e-diary data online at frequent intervals as part of the ongoing safety review.

The investigator or designee must contact the subject in order to obtain stop dates for any ongoing local reactions or systemic events on the last day that the e-diary was completed. The stop dates should be documented in the source documents and the information entered in the CRF.

7.2.1.1. Grading Scales

The grading scales used in this study to assess local reactions and systemic events as described below are derived from the FDA Center for Biologics Evaluation and Research (CBER) guidelines on toxicity grading scales for healthy adult volunteers enrolled in preventive vaccine clinical trials.[62]

7.2.1.2. Local Reactions

During the e-diary reporting period, subjects will be asked to assess redness, swelling, and pain at the injection site and to record the symptoms in the e-diary. If a local reaction persists beyond the end of the e-diary period following vaccination, the subject will be requested to report that information. The investigator will enter this additional information in the CRF.

Redness and swelling will be measured and recorded in measuring device units (range: 1 to 21) and then categorized during analysis as absent, mild, moderate, or severe based on the grading scale in Table 3. Measuring device units can be converted to centimeters according to the following formula: 1 measuring device unit = 0.5 cm.

Pain at the vaccine injection site will be assessed by the subject as absent, mild, moderate, or severe according the grading scale in Table 3.

If a Grade 3 local reaction is reported in the e-diary, a telephone contact should occur to ascertain further details and determine whether a site visit is clinically indicated. Only an investigator or medically qualified person is able to classify a subject's local reaction as Grade 4. If a subject experiences a confirmed Grade 4 local reaction, the investigator must immediately notify the sponsor and, if it is determined to be related to the administration of the investigational product, further vaccinations will be discontinued in that subject.

Table 3. Local Reaction Grading Scale

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

7.2.1.3. Systemic Events

During the e-diary reporting period, subjects will be asked to assess vomiting, headache, fatigue, new or worsening muscle pain, and new or worsening joint pain and to record the symptoms in the e-diary. The symptoms will be assessed by the subject as absent, mild, moderate, or severe according to the grading scale in Table 4.

If a Grade 3 systemic event is reported in the e-diary, a telephone contact should occur to ascertain further details and determine whether a site visit is clinically indicated. Only an investigator or medically qualified person is able to classify a subject's systemic event as Grade 4. If a subject experiences a confirmed Grade 4 systemic event, the investigator must immediately notify the sponsor and, if it is determined to be related to the administration of the investigational product, further vaccinations will be discontinued in that subject.

Table 4. Systemic Event Grading Scale

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Vomiting	1-2 times in 24 hours	>2 times in 24 hours	Requires IV hydration	Emergency room visit or hospitalization for hypotensive shock

Table 4. Systemic Event Grading Scale

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Headache	No interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency room visit or hospitalization for severe headache
Fatigue/ Tiredness	No interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency room visit or hospitalization for severe fatigue
New or worsening muscle pain	No interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency room visit or hospitalization for severe new or worsening muscle pain
New or worsening joint pain	No interference with activity	Some interference with activity	Significant; prevents daily activity	Emergency room visit or hospitalization for severe new or worsening joint pain

Abbreviation: IV = intravenous.

7.2.1.4. Fever

In order to record information on fever, a digital thermometer will be given to subjects with instructions on how to measure oral temperature at home. Temperature will be collected in the e-diary in the evening daily during the e-diary reporting period. It will also be collected at any time during the e-diary data collection periods when fever is suspected. 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 measured and recorded to 1 decimal place and then categorized during analysis according to the scale shown in Table 5.

If a Grade 3 fever is reported in the e-diary, a telephone contact should occur to ascertain further details and determine whether a site visit is clinically indicated. Only an investigator or medically qualified person is able to classify a subject's fever as Grade 4. If a subject experiences a confirmed Grade 4 fever, the investigator must immediately notify the sponsor and, if it is determined to be related to the administration of the investigational product, further vaccinations will be discontinued in that subject.

Table 5. Scale for Fever

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

CCI [redacted]

7.4. Other Assessments

At the time of a diarrheal episode, the site will complete a questionnaire with the subject to collect information on severity, duration, treatment of, and healthcare utilization associated with the episode. CCI [redacted]
CCI [redacted]

CCI [redacted]

7.5. Pregnancy Testing

For female subjects of childbearing potential, a urine pregnancy test, with sensitivity of at least 25 mIU/mL, will be performed immediately before administration of each vaccine dose. A negative pregnancy test result is required before the subject may receive the investigational product. Pregnancy tests may also be repeated if requested by institutional review boards (IRBs)/ethics committees (ECs) or if required by local regulations. In the case of a positive confirmed pregnancy, the subject will be withdrawn from administration of investigational product but may remain in the study.

Urine pregnancy tests must be sensitive to at least 25 mIU/mL and will be conducted with the test kit provided by the sponsor in accordance with instructions provided in its package insert. Subjects who have missed a menstrual period or who show an indeterminate or positive result on the qualitative point-of-service urine test may not further progress in the study until pregnancy is ruled out using further diagnostic testing (eg, a negative quantitative serum pregnancy test conducted at a certified laboratory).

CCI

[redacted]

8. ADVERSE EVENT REPORTING

8.1. Requirements

The table below summarizes the requirements for recording safety events on the CRF and for reporting safety events on the Clinical Trial (CT) Serious Adverse Event (SAE) Report Form to Pfizer Safety. These requirements are delineated for 3 types of events: (1) SAEs; (2) nonserious AEs; and (3) exposure to the investigational product under study during pregnancy or breastfeeding, and occupational exposure.

Safety Event	**Recorded on the CRF**	**Reported on the CT SAE Report Form to Pfizer Safety Within 24 Hours of Awareness**
SAE	All	All
Nonserious AE	All	None
Exposure to the investigational product under study during pregnancy or breastfeeding, and occupational exposure	All (regardless of whether associated with an AE), **except occupational exposure**	Exposure during pregnancy, exposure via breastfeeding, occupational exposure (regardless of whether associated with an AE)

All observed or volunteered events regardless of vaccine group or suspected causal relationship to the investigational product(s) will be reported as described in the following paragraphs.

Events listed in the table above that require reporting to Pfizer Safety on the CT SAE Report Form within 24 hours of awareness of the event by the investigator **are to be reported regardless of whether the event is determined by the investigator to be related to an investigational product under study**. In particular, if the SAE is fatal or life-threatening, notification to Pfizer Safety must be made immediately, irrespective of the extent of available event information.

This time frame also applies to additional new (follow-up) information on previously forwarded reports. In the rare situation that the investigator does not become immediately aware of the occurrence of an event, the investigator must report the event within 24 hours after learning of it and document the time of his/her first awareness of the event.

For each event, the investigator must pursue and obtain adequate information both to determine the outcome and to assess whether it meets the criteria for classification as an SAE (see the Serious Adverse Events section below). In addition, the investigator may be requested by Pfizer Safety to obtain specific follow-up information in an expedited fashion. This information is more detailed than that recorded on the CRF. In general, this will include a description of the event in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Any information relevant to the event, such as concomitant medications and illnesses, must be provided. In the case of a subject death, a summary of available autopsy findings must be submitted as soon as possible to Pfizer Safety. Any pertinent additional information must be reported on the CT SAE Report Form; additional source documents (eg, medical records, CRF, laboratory data) are to be sent to Pfizer Safety **ONLY** upon request.

As part of ongoing safety reviews conducted by the sponsor, any nonserious AE that is determined by the sponsor to be serious will be reported by the sponsor as an SAE. To assist in the determination of case seriousness, further information may be requested from the investigator to provide clarity and understanding of the event in the context of the clinical study.

8.1.1. Additional Details on Recording Adverse Events on the CRF

All events detailed in the table above will be recorded on the AE page(s) of the CRF. It should be noted that the CT SAE Report Form for reporting of SAE information is not the same as the AE page of the CRF. When the same data are collected, the forms must be completed in a consistent manner. AEs should be recorded using concise medical terminology, and the same AE term should be used on both the CRF and the CT SAE Report Form for reporting of SAE information.

8.1.2. Eliciting Adverse Event Information

The investigator is to record on the CRF all directly observed AEs and all AEs spontaneously reported by the study subject. In addition, each study subject will be questioned about the occurrence of AEs in a nonleading manner.

8.1.3. Withdrawal From the Study Due to Adverse Events (see also the Subject Withdrawal section)

Withdrawal due to AEs should be distinguished from withdrawal due to other causes, according to the definition of AE noted below, and recorded on the CRF.

When a subject withdraws from the study because of an SAE, the SAE must be recorded on the CRF and reported, as appropriate, on the CT SAE Report Form, in accordance with the Requirements section above.

8.1.4. Time Period for Collecting AE/SAE Information

The time period for actively eliciting and collecting AEs and SAEs ("active collection period") for each subject begins from the time the subject provides informed consent, which is obtained before the subject's participation in the study (ie, before undergoing any study-related procedure and/or receiving investigational product). All AEs are collected through and including Visit 5 (Month 7). Additionally, SAEs are collected between Visit 5 (Month 7) through Visit 6 (Month 12), as elicited by telephone.

In addition, any AE occurring up to 48 hours after each subsequent blood draw (ie, after Visit 5 [Month 7] and at the time of a diarrheal episode) must be recorded on the CRF. Diarrheal episode AEs must also be recorded on the CRF throughout the study.

For subjects who are screen failures, the active collection period ends when screen failure status is determined.

8.1.4.1. Reporting SAEs to Pfizer Safety

All SAEs occurring in a subject during the active collection period are reported to Pfizer Safety on the CT SAE Report Form.

SAEs occurring in a subject after the active collection period has ended are reported to Pfizer Safety if the investigator becomes aware of them; at a minimum, all SAEs that the investigator believes have at least a reasonable possibility of being related to investigational product must be reported to Pfizer Safety. If an SAE is reported after subject Visit 6, all the requirements will apply, including the respect of the reporting timelines as if the SAE occurred within the reporting period.

Follow-up by the investigator continues throughout and after the active collection period and until the event or its sequelae resolve or stabilize at a level acceptable to the investigator, and Pfizer concurs with that assessment.

8.1.4.2. Recording Nonserious AEs and SAEs on the CRF

During the active collection period, both nonserious AEs and SAEs are recorded on the CRF.

Follow-up by the investigator may be required until the event or its sequelae resolve or stabilize at a level acceptable to the investigator, and Pfizer concurs with that assessment.

8.1.5. Causality Assessment

The investigator's assessment of causality must be provided for all AEs (serious and nonserious); the investigator must record the causal relationship on the CRF, and report such an assessment in accordance with the SAE reporting requirements, if applicable. An investigator's causality assessment is the determination of whether there exists a reasonable possibility that the investigational product caused or contributed to an AE; generally the facts (evidence) or arguments to suggest a causal relationship should be provided.

If the investigator does not know whether or not the investigational product caused the event, then the event will be handled as "related to investigational product" for reporting purposes, as defined by the sponsor. If the investigator's causality assessment is "unknown but not related" to investigational product, this should be clearly documented on study records.

In addition, if the investigator determines that an SAE is associated with study procedures, the investigator must record this causal relationship in the source documents and CRF, and report such an assessment in the dedicated section of the CT SAE Report Form and in accordance with the SAE reporting requirements.

8.1.6. Sponsor's Reporting Requirements to Regulatory Authorities

AE reporting, including suspected unexpected serious adverse reactions, will be carried out in accordance with applicable local regulations.

8.2. Definitions

8.2.1. Adverse Events

An AE is any untoward medical occurrence in a study subject administered a product or medical device; the event need not necessarily have a causal relationship with the treatment or usage. Examples of AEs include, but are not limited to:

- Abnormal test findings;
- Clinically significant signs and symptoms;
- Changes in physical examination findings;
- Hypersensitivity;
- Progression/worsening of underlying disease;
- Drug abuse;
- Drug dependency.

Additionally, AEs may include signs and symptoms resulting from:

- Drug overdose;
- Drug withdrawal;
- Drug misuse;
- Drug interactions;
- Extravasation;

- Exposure during pregnancy (EDP);
- Exposure via breastfeeding;
- Medication error;
- Occupational exposure.

8.2.2. Abnormal Test Findings

Abnormal objective test findings should be recorded as AEs when any of the following conditions are met:

- Test result is associated with accompanying symptoms; and/or
- Test result requires additional diagnostic testing or medical/surgical intervention; and/or
- Test result leads to a change in study dosing (outside of any protocol-specified dose adjustments) or discontinuation from the study, significant additional concomitant drug treatment, or other therapy; and/or
- Test result is considered to be an AE by the investigator or sponsor.

Merely repeating an abnormal test, in the absence of any of the above conditions, does not constitute an AE. Any abnormal test result that is determined to be an error does not require recording as an AE.

8.2.3. Serious Adverse Events

An SAE is any untoward medical occurrence at any dose that:

- Results in death;
- Is life-threatening (immediate risk of death);
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity (substantial disruption of the ability to conduct normal life functions);
- Results in congenital anomaly/birth defect.

Or that is considered to be:

- An important medical event.

Medical and scientific judgment is exercised in determining whether an event is an important medical event. An important medical event may not be immediately life-threatening and/or result in death or hospitalization. However, if it is determined that the event may jeopardize the subject or may require intervention to prevent one of the other AE outcomes, the important medical event should be reported as serious.

Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse.

Medical device complaints may meet the SAE reporting requirement criteria (see the Medical Device Complaint Reporting Requirements section). An incident is any malfunction (ie, the failure of a device to meet its performance specifications or to perform as intended; performance specifications include all claims made in the labeling for the device) that, directly or indirectly, might lead to or might have led to the death of a subject, or user, or of other persons, or to a serious deterioration in their state of health.

A serious injury that can cause a serious deterioration in state of health can include:

- A life-threatening illness, even if temporary in nature;
- A permanent impairment of a body function or permanent damage to a body structure;
- A condition necessitating medical or surgical intervention to prevent the above 2 bulleted items;

  Examples: clinically relevant increase in the duration of a surgical procedure; a condition that requires hospitalization or significant prolongation of existing hospitalization;
- Any indirect harm as a consequence of an incorrect diagnostic or in vitro diagnostic device test results when used within the manufacturer's instructions for use;
- Fetal distress, fetal death, or any congenital abnormality or birth defects.

8.2.4. Hospitalization

Hospitalization is defined as any initial admission (even less than 24 hours) in a hospital or equivalent healthcare facility, or any prolongation of an existing admission. Admission also includes transfer within the hospital to an acute/intensive care unit (eg, from the psychiatric wing to a medical floor, medical floor to a coronary care unit, or neurological floor to a tuberculosis unit). An emergency room visit does not necessarily constitute a hospitalization; however, the event leading to the emergency room visit is assessed for medical importance.

Hospitalization does not include the following:

- Rehabilitation facilities;
- Hospice facilities;
- Respite care (eg, caregiver relief);
- Skilled nursing facilities;
- Nursing homes;
- Same-day surgeries (as outpatient/same-day/ambulatory procedures).

Hospitalization or prolongation of hospitalization in the absence of a precipitating clinical AE is not in itself an SAE. Examples include:

- Admission for treatment of a preexisting condition not associated with the development of a new AE or with a worsening of the preexisting condition (eg, for workup of a persistent pretreatment laboratory abnormality);
- Social admission (eg, subject has no place to sleep);
- Administrative admission (eg, for yearly physical examination);
- Protocol-specified admission during a study (eg, for a procedure required by the study protocol);
- Optional admission not associated with a precipitating clinical AE (eg, for elective cosmetic surgery);
- Hospitalization for observation without a medical AE;
- Preplanned treatments or surgical procedures. These should be noted in the baseline documentation for the entire protocol and/or for the individual subject.

Diagnostic and therapeutic noninvasive and invasive procedures, such as surgery, should not be reported as SAEs. However, the medical condition for which the procedure was performed should be reported if it meets the definition of an SAE. For example, an acute appendicitis that begins during the reporting period should be reported if the SAE requirements are met, and the resulting appendectomy should be recorded as treatment of the AE.

8.3. Severity Assessment

GRADE	If required on the AE page of the CRF, the investigator will use the adjectives MILD, MODERATE, SEVERE, or LIFE-THREATENING to describe the maximum intensity of the AE. For purposes of consistency, these intensity grades are defined as follows:	
1	MILD	Does not interfere with subject's usual function.
2	MODERATE	Interferes to some extent with subject's usual function.
3	SEVERE	Interferes significantly with subject's usual function.
4	LIFE-THREATENING	Life-threatening consequences; urgent intervention indicated.

Note the distinction between the severity and the seriousness of an AE. A severe event is not necessarily an SAE. For example, a headache may be severe (interferes significantly with the subject's usual function) but would not be classified as serious unless it met one of the criteria for SAEs, listed above.

8.4. Special Situations

8.4.1. Protocol-Specified Serious Adverse Events

There are no protocol-specified SAEs in this study. All SAEs will be reported to Pfizer Safety by the investigator as described in previous sections, and will be handled as SAEs in the safety database.

8.4.2. Potential Cases of Drug-Induced Liver Injury

Humans exposed to a drug who show no sign of liver injury (as determined by elevations in transaminases) are termed “tolerators,” while those who show transient liver injury, but adapt are termed “adaptors.” In some subjects, transaminase elevations are a harbinger of a more serious potential outcome. These subjects fail to adapt and therefore are "susceptible" to progressive and serious liver injury, commonly referred to as drug-induced liver injury (DILI). Subjects who experience a transaminase elevation above 3 times the upper limit of normal (× ULN) should be monitored more frequently to determine if they are an “adaptor” or are “susceptible.”

Liver function tests (LFTs) are not required as a routine safety monitoring procedure in this study. However, should an investigator deem it necessary to assess LFTs because a subject presents with clinical signs/symptoms, such LFT results should be managed and followed as described below.

In the majority of DILI cases, elevations in aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) precede total bilirubin (TBili) elevations (>2 × ULN) by several days or weeks. The increase in TBili typically occurs while AST/ALT is/are still elevated above 3 × ULN (ie, AST/ALT and TBili values will be elevated within the same laboratory sample). In rare instances, by the time TBili elevations are detected, AST/ALT values might have decreased. This occurrence is still regarded as a potential DILI. Therefore, abnormal

elevations in either AST OR ALT in addition to TBili that meet the criteria outlined below are considered potential DILI (assessed per Hy's law criteria) cases and should always be considered important medical events, even before all other possible causes of liver injury have been excluded.

The threshold of laboratory abnormalities for a potential DILI case depends on the subject's individual baseline values and underlying conditions. Subjects who present with the following laboratory abnormalities should be evaluated further as potential DILI (Hy's law) cases to definitively determine the etiology of the abnormal laboratory values:

- Subjects with AST/ALT and TBili baseline values within the normal range who subsequently present with AST OR ALT values >3 × ULN AND a TBili value >2 × ULN with no evidence of hemolysis and an alkaline phosphatase value <2 × ULN or not available;

- For subjects with baseline AST **OR** ALT **OR** TBili values above the ULN, the following threshold values are used in the definition mentioned above, as needed, depending on which values are above the ULN at baseline:

    - Preexisting AST or ALT baseline values above the normal range: AST or ALT values >2 times the baseline values AND >3 × ULN; or >8 × ULN (whichever is smaller).

    - Preexisting values of TBili above the normal range: TBili level increased from baseline value by an amount of at least 1 × ULN **or** if the value reaches >3 × ULN (whichever is smaller).

Rises in AST/ALT and TBili separated by more than a few weeks should be assessed individually based on clinical judgment; any case where uncertainty remains as to whether it represents a potential Hy's law case should be reviewed with the sponsor.

The subject should return to the investigator site and be evaluated as soon as possible, preferably within 48 hours from awareness of the abnormal results. This evaluation should include laboratory tests, detailed history, and physical assessment.

In addition to repeating measurements of AST and ALT and TBili, laboratory tests should include albumin, creatine kinase (CK), direct and indirect bilirubin, gamma-glutamyl transferase (GGT), prothrombin time (PT)/international normalized ratio (INR), total bile acids, alkaline phosphatase, and acetaminophen drug and/or protein adduct levels. Consideration should also be given to drawing a separate tube of clotted blood and an anticoagulated tube of blood for further testing, as needed, for further contemporaneous analyses at the time of the recognized initial abnormalities to determine etiology. A detailed history, including relevant information, such as review of ethanol, acetaminophen (either by itself or as a coformulated product in prescription or over-the-counter medications), recreational drug, supplement (herbal) use and consumption, family history, sexual history, travel history, history of contact with a jaundiced person, surgery, blood transfusion, history of liver or allergic disease, and potential occupational exposure to chemicals, should be

collected. Further testing for acute hepatitis A, B, C, D, and E infection and liver imaging (eg, biliary tract) may be warranted.

All cases demonstrated on repeat testing as meeting the laboratory criteria of AST/ALT and TBili elevation defined above should be considered potential DILI (Hy's law) cases if no other reason for the LFT abnormalities has yet been found. **Such potential DILI (Hy's law) cases are to be reported as SAEs, irrespective of availability of all the results of the investigations performed to determine etiology of the LFT abnormalities.**

A potential DILI (Hy's law) case becomes a confirmed case only after all results of reasonable investigations have been received and have excluded an alternative etiology.

8.4.3. Exposure to the Investigational Product During Pregnancy or Breastfeeding, and Occupational Exposure

Exposure to the investigational product under study during pregnancy or breastfeeding and occupational exposure are reportable to Pfizer Safety within 24 hours of investigator awareness.

8.4.3.1. Exposure During Pregnancy

For both unapproved/unlicensed products and for marketed products, an EDP occurs if:

- A female becomes, or is found to be, pregnant either while receiving or having been exposed (eg, because of treatment or environmental exposure) to the investigational product; or the female becomes or is found to be pregnant after discontinuing and/or being exposed to the investigational product;
    - An example of environmental exposure would be a case involving direct contact with a Pfizer product in a pregnant woman (eg, a nurse reports that she is pregnant and has been exposed to chemotherapeutic products).
- A male has been exposed (eg, because of treatment or environmental exposure) to the investigational product prior to or around the time of conception and/or is exposed during his partner's pregnancy.

If a subject or subject's partner becomes or is found to be pregnant during the subject's treatment with the investigational product, the investigator must report this information to Pfizer Safety on the CT SAE Report Form and an EDP supplemental form, regardless of whether an SAE has occurred. In addition, the investigator must submit information regarding environmental exposure to a Pfizer product in a pregnant woman (eg, a subject reports that she is pregnant and has been exposed to a cytotoxic product by inhalation or spillage) to Pfizer Safety using the EDP supplemental form. This must be done irrespective of whether an AE has occurred and within 24 hours of awareness of the exposure. The information submitted should include the anticipated date of delivery (see below for information related to termination of pregnancy).

Follow-up is conducted to obtain general information on the pregnancy and its outcome for all EDP reports with an unknown outcome. The investigator will follow the pregnancy until completion (or until pregnancy termination) and notify Pfizer Safety of the outcome as a follow-up to the initial EDP supplemental form.

In the case of a live birth, the structural integrity of the neonate can be assessed at the time of birth. In the event of a termination, the reason(s) for termination should be specified and, if clinically possible, the structural integrity of the terminated fetus should be assessed by gross visual inspection (unless preprocedure test findings are conclusive for a congenital anomaly and the findings are reported).

If the outcome of the pregnancy meets the criteria for an SAE (ie, ectopic pregnancy, spontaneous abortion, intrauterine fetal demise, neonatal death, or congenital anomaly [in a live-born baby, a terminated fetus, an intrauterine fetal demise, or a neonatal death]), the investigator should follow the procedures for reporting SAEs.

Additional information about pregnancy outcomes that are reported to Pfizer Safety as SAEs follows:

- Spontaneous abortion includes miscarriage and missed abortion;
- Neonatal deaths that occur within 1 month of birth should be reported, without regard to causality, as SAEs. In addition, infant deaths after 1 month should be reported as SAEs when the investigator assesses the infant death as related or possibly related to exposure to the investigational product.

Additional information regarding the EDP may be requested by the sponsor. Further follow-up of birth outcomes will be handled on a case-by-case basis (eg, follow-up on preterm infants to identify developmental delays). In the case of paternal exposure, the investigator will provide the subject with the Pregnant Partner Release of Information Form to deliver to his partner. The investigator must document in the source documents that the subject was given the Pregnant Partner Release of Information Form to provide to his partner.

8.4.3.2. Exposure During Breastfeeding

Scenarios of exposure during breastfeeding must be reported, irrespective of the presence of an associated SAE, to Pfizer Safety within 24 hours of the investigator's awareness, using the CT SAE Report Form. An exposure during breastfeeding report is not created when a Pfizer drug specifically approved for use in breastfeeding women (eg, vitamins) is administered in accord with authorized use. However, if the infant experiences an SAE associated with such a drug's administration, the SAE is reported together with the exposure during breastfeeding.

8.4.3.3. Occupational Exposure

An occupational exposure occurs when, during the performance of job duties, a person (whether a healthcare professional or otherwise) gets in unplanned direct contact with the product, which may or may not lead to the occurrence of an AE.

An occupational exposure is reported to Pfizer Safety within 24 hours of the investigator's awareness, using the CT SAE Report Form, regardless of whether there is an associated SAE. Since the information does not pertain to a subject enrolled in the study, the information is not recorded on a CRF; however, a copy of the completed CT SAE Report Form is maintained in the investigator site file.

8.4.4. Medication Errors

Other exposures to the investigational product under study may occur in clinical trial settings, such as medication errors.

Safety Event	**Recorded on the CRF**	**Reported on the CT SAE Report Form to Pfizer Safety Within 24 Hours of Awareness**
Medication errors	All (regardless of whether associated with an AE)	Only if associated with an SAE

8.4.4.1. Medication Errors

Medication errors may result from the administration or consumption of the investigational product by the wrong subject, or at the wrong time, or at the wrong dosage strength.

Medication errors include:

- Medication errors involving subject exposure to the investigational product;
- Potential medication errors or uses outside of what is foreseen in the protocol that do or do not involve the participating subject.

Other examples include, but are not limited to:

- The administration of expired investigational product;
- The administration of an incorrect investigational product;
- The administration of an incorrect dosage;
- The administration of investigational product that has undergone temperature excursion from the specified storage range, unless it is determined by the sponsor that the investigational product under question is acceptable for use.

Such medication errors occurring to a study subject are to be captured on the medication error page of the CRF, which is a specific version of the AE page.

In the event of a medication dosing error, the sponsor should be notified immediately.

Whether or not the medication error is accompanied by an AE, as determined by the investigator, the medication error is recorded on the medication error page of the CRF and, if applicable, any associated AE(s), serious and nonserious, are recorded on an AE page of the CRF.

Medication errors should be reported to Pfizer Safety within 24 hours on a CT SAE Report Form **only when associated with an SAE**.

8.5. Medical Device Complaint Reporting Requirements

All medical device complaints, regardless of whether the medical device complaint is associated with an AE, will be reported by a member of unblinded site staff as described in the IP manual. This includes potential incidents or malfunctions associated with the use of a medical device product. An incident or malfunction is an event that might have led to death or serious deterioration in health, or if it occurred again might lead to death or serious deterioration in health.

Pfizer is to be notified of all medical device complaints within 24 hours of the investigator's awareness of the event.

9. DATA ANALYSIS/STATISTICAL METHODS

The methodology for summary and statistical analyses of the data collected in this study is outlined here and further detailed in a statistical analysis plan (SAP), which will be maintained by the sponsor. The SAP may modify what is outlined in the protocol where appropriate; however, any major modifications of the primary endpoint definitions or their analyses will also be reflected in a protocol amendment. Supplemental analyses of the primary, secondary, immunogenicity, and safety data may be performed as required for local regulatory jurisdictions (for example, analyses by age, sex, ethnicity, risk status prior to vaccination) and will be prespecified in the SAP.

The SAP will be finalized prior to any analyses being undertaken.

9.1. Sample Size Determination

This study is placebo-controlled, event-driven, and randomized with a 1:1 randomization ratio between subjects receiving *C difficile* vaccine and placebo.

With assumptions of a true adjusted VE of 66.5% after 3 doses of investigational product, 66 first primary CDI cases (meeting case definition 1 in the per-protocol-3 population Section 2.1.1 and Section 9.2.1) will provide 89.8% power to conclude true VE of >20%, taking into consideration 3 interim analyses (Section 9.5) during the study.

The early efficacy stopping boundaries are based on a GS design[65] utilizing a single-binomial distribution, conditional on a total of 66 cases, assuming true VE of 66.5%, with 3 interim analyses at 30, 40 and 50 cases.[65] The same gamma[-2] alpha spending function that was used for the original statistical design is also used for this modified statistical design.

As this is an event-driven and GS study, the total enrollment number may vary depending on the assumptions of incidence rate of the primary endpoint, true VE, and potential early stop for efficacy or futility.

Update as Part of Amendment 2 (January 2019):
Based on the ongoing blinded review of pooled primary CDI events, the annual CDI incidence rate in the placebo group may be different from the assumed rate in the power calculation during the initial study design. Additional enrollment may be needed in order to maintain the study power of 90% (106 first primary CDI cases in the final analysis) to reject the null hypothesis of VE ≤20%. Assuming a true VE of 60% after 3 doses of investigational product and the true annual CDI rate in the placebo group of 0.552% to 0.516%, which is 8 to 14% lower than the previously assumed 0.6%, an additional 1369 to 2566 subjects may be needed to ensure the required number of first primary CDI cases.

In light of this, it is planned to enroll approximately 1700 additional subjects, giving a total of approximately 17,476 subjects. The pooled primary CDI event rate for the total population will continue to be monitored by the study team in a blinded manner and a further adjustment to enrollment may be required.

Update as Part of Amendment 4 (July 2020):
The statistical design is being modified to perform all the analyses with fewer cases than originally planned. Two (2) additional interim analyses will be included based on the estimated disease rate from the blinded data review, and the assumption regarding true VE is modified to 68%, and is further adjusted to 66.5% after 3 doses of investigational product because of the impact of actual specificity (99.1%) and sensitivity (95.7%) of Pfizer's cell cytotoxicity neutralization assay.[66]

The pooled primary CDI event rate for the total population will continue to be monitored by the study team in a blinded manner and a further adjustment to enrollment may be required.

As specified in Section 3.1, the pooled primary CDI event rates in different age groups, inclusion criteria subgroups, CCI[61] subgroups, and other baseline factors will be monitored in a blinded manner. Once the specified minimum numbers in each age group have been achieved, if a substantially higher pooled CDI event rate is observed in some subgroups, the intention may be to stop enrollment in lower-risk subgroups and complete enrollment with the higher-risk subgroups thereafter. This is mainly to help to achieve the targeted total number of first primary cases in a timely manner. As none of the potential adjustments will be based on the observed VE of the ongoing study, and the targeted total number of first primary CDI cases will remain the same, the distribution of the test statistic for the primary endpoint under the null hypothesis will not be affected and no alpha adjustment will be required.

For the second primary hypothesis test, it is expected that more than 66 cases for the final analysis will occur after 2 or more doses because of the nature of the endpoint. Any additional first primary CDI cases occurring in the interval between Doses 2 and 3 will result in greater than 90% power to conclude true VE of >20% after 2 or more doses of vaccine assuming the vaccine first successfully demonstrates VE of >20% following 3 doses of vaccine.

9.2. Efficacy Analysis

All of the following VE analyses assume correct classification of suspected CDI cases for all case definitions.

Five (5) analysis populations (per-protocol-2, per-protocol-3, mITT-1, mITT-2, and mITT-3) will be defined for VE analyses.

- The per-protocol-2 population will include all randomized subjects who received at least 2 doses of the investigational product to which they were randomized and had no major protocol violations up to and including 14 days after Dose 2.
- The per-protocol-3 population will include all randomized subjects who received all 3 doses of the investigational product to which they were randomized and had no major protocol violations up to and including 14 days after Dose 3.
- The mITT-1 population will include all randomized subjects who received at least 1 dose of investigational product.
- The mITT-2 population will include all randomized subjects who received at least 2 doses of investigational product.
- The mITT-3 population will include all randomized subjects who received all 3 doses of investigational product.

The per-protocol-2 and per-protocol-3 populations will be the primary populations for efficacy analyses accounting for the time interval after dosing as given in Table 6 and will be used for analyzing VE endpoints. The mITT-1, mITT-2, and mITT-3 populations will be supportive populations for efficacy analyses and will be used for analyzing VE endpoints from immediately or 14 days after receipt of each dose of investigational product.

9.2.1. Analysis of the Primary Endpoints

VE is defined as VE = 100 × (1 – IRR), where IRR is the infection rate ratio, the calculated ratio of first primary CDI incidence between the *C difficile* vaccine group and the placebo group. VE_i will be evaluated separately after 3 and after 2 doses. The 2 primary efficacy hypotheses are (in order of fixed-sequence testing):

1. H_{03}: $VE_3 \leq 20\%$ vs H_{a3}: $VE_3 > 20\%$, and
2. H_{02}: $VE_2 \leq 20\%$ vs H_{a2}: $VE_2 > 20\%$.

where H_{0i} and H_{ai} represent null hypothesis and alternative hypothesis, and VE_i (i = 2, 3) represents VE after at least 2 doses and after all 3 doses, respectively. For subjects with multiple primary CDI cases, only the first primary case will contribute to the VE calculation in each hypothesis test.

The primary efficacy hypotheses will be tested by a 1-sided test for VE >20% by a fixed-sequence testing procedure, with testing order of H_{03} followed by H_{02} using the approach described in Chan and Bohidar,[63] accounting for follow-up time after vaccination. Statistical inference will be based on the lower alpha-adjusted confidence interval (CI) for VE_3. and VE_2.

If the study continues after all 3 of the interim analyses, once 66 first primary cases after 3 doses have accumulated for the final analysis, the hypothesis test H_{03} will be performed first, and the null hypothesis H_{03} will be rejected if there are 20 or fewer cases observed in the *C difficile* vaccine group (corresponding to a 2-sided lower bound of 96.4% exact CI >20%), assuming equal follow-up times. The lower bound of the 2-sided 96.4% exact CI corresponds to α=0.018 (one-sided) used for final testing in a GS design with a gamma(-2) efficacy boundary. Only if the hypothesis H_{03} is rejected, then hypothesis testing for H_{02} will be performed, at the same significance level for VE_2 for the final analysis. The statistical success criterion for H_{02} also corresponds to the lower bound of the 2-sided 96.4% CI for VE being >20%. If there is failure to reject the hypothesis H_{03}, the hypothesis test H_{02} will not be performed.

The statistical analysis specifications for the primary endpoints are summarized in Table 6.

Table 6. Statistical Analysis Specifications for the Primary Endpoints

Primary Endpoints	Time Period for Case Accrual	CDI Case Definition	Analysis Population	Hypothesis Testing[a]	Statistical Testing Method
CDI incidence of a first primary episode per 1000 person-years of follow-up	From 14 days after receipt of third dose of investigational product onwards	1	Per-protocol-3 (primary)	H_{03}	Exact binomial, incidence rate
CDI incidence of a first primary episode per 1000 person-years of follow-up	From 14 days after receipt of third dose of investigational product onwards	1	mITT-3 (supportive)	H_{03}	Exact binomial, incidence rate
CDI incidence of a first primary episode per 1000 person-years of follow-up	From immediately after receipt of third dose of investigational product onwards	1	mITT-3 (supportive)	H_{03}	Exact binomial, incidence rate
CDI incidence of a first primary episode per 1000 person-years of follow-up	From 14 days after receipt of second dose of investigational product onwards	1	Per-protocol-2 (primary)	H_{02}	Exact binomial, incidence rate

Table 6. Statistical Analysis Specifications for the Primary Endpoints

Primary Endpoints	Time Period for Case Accrual	CDI Case Definition	Analysis Population	Hypothesis Testing[a]	Statistical Testing Method
CDI incidence of a first primary episode per 1000 person-years of follow-up	From 14 days after receipt of second dose of investigational product onwards	1	mITT-2 (supportive)	H_{02}	Exact binomial, incidence rate
CDI incidence of a first primary episode per 1000 person-years of follow-up	From immediately after receipt of second dose of investigational product onwards	1	mITT-2 (supportive)	H_{02}	Exact binomial, incidence rate

Abbreviations: CDI = *Clostridium difficile* infection; mITT = modified intent-to-treat.

a. For the per-protocol populations, hypotheses H_{02} and H_{03} will be tested in the fixed order of H_{03} followed by H_{02}.

9.2.1.1. Multiplicity Control for the Primary Endpoints

There are 2 primary efficacy hypotheses; H_{03}, followed by H_{02}, if H_{03} is rejected. The study will be considered successful if H_{03} is rejected.

There are 2 sources of multiplicity in the study. First, there are repeated analyses of the primary hypothesis H_{03}, at the interim and final analyses. Alpha adjustment due to the planned interim analyses is explained in Section 9.5. The second source of multiplicity is the existence of multiple primary hypotheses. The overall type I error rate for all primary hypotheses is controlled by the fixed -sequence testing procedure as described in Section 9.2.1. Testing hypothesis H_{02} will be considered as final analysis at the type I error of 0.018 only if H_{03} is rejected and a decision is made to proceed to the final analysis.

9.2.2. Analysis of the Secondary Endpoints

9.2.2.1. Analysis of the Secondary Efficacy Endpoints

The analyses of the secondary efficacy endpoints will be performed based on a variety of CDI case definitions, detailed in Section 2.1, after the primary objectives are met. For the endpoints that combine data for subjects who fulfill case definition 1 and subjects who fulfill case definition 3, or case definitions 2 and 4, each subject will contribute only once to the analysis.

In the evaluation of the efficacy of 2 doses of *C difficile* vaccine, only cases starting after the receipt of the second dose but prior to the receipt of the third dose of investigational product will contribute to the VE calculation.

In order to control overall Type I error, and specifically the Type I error for the evaluation of the secondary objectives, an alpha level of 0.018 (one-sided) for the final analysis is split equally (the Bonferroni correction, alpha = 0.009, for each family evaluation) for the evaluation of the secondary objectives between the 3-dose family and the 2-dose family (at-least-2-dose/only-2-dose), after primary objectives are met.

Furthermore, within each family, the endpoints will be evaluated sequentially; that is, the subsequent endpoint is evaluated only if the criteria are met for the previous endpoints.

The analysis and the success criteria for the secondary objective evaluation are outlined below:

- For evaluating *C difficile* vaccine in reducing the incidence of CDI cases, the lower limit (LL) of 98.2% CI (2-sided) for VE > 0.
- For evaluating the efficacy of Pfizer's *C difficile* vaccine in reducing the severity of CDI in terms of the duration, the upper limit (UL) of 98.2% CI (2-sided) < 0 for the difference in mean duration of CDI between the vaccine group and the control group.
- For evaluating the efficacy of Pfizer's *C difficile* vaccine in reducing the severity of CDI in terms of the risk ratio, the UL of 98.2% CI (2-sided) < 1.0 for the ratio of the proportion of subjects experiencing CDI who have a non-protocol-related medically attended visit in the vaccine group to the proportion of the subjects in the control group.

CCI

CCI

[redacted]

[redacted]

[redacted]

9.4. Safety Analysis

The safety population will include all subjects who receive at least 1 dose of investigational product. The safety population for reactogenicity analysis after each dose will include subjects who received that dose and have e-diary information available. For safety analysis, subjects will be analyzed according to the investigational product received.

AEs will be categorized according to Medical Dictionary for Regulatory Activities (MedDRA) and will be summarized by vaccine group. All summaries of AEs will show the number and percentage of subjects experiencing at least 1 event and the number of events for each vaccine group. Two (2)-sided 95% CIs based on the Clopper-Pearson method will be presented for (S)AE percentages.

A 3-tier approach will be used to summarize AEs. Under this approach, AEs are classified into 1 of 3 tiers:

- Tier 1 events: These are prespecified events of clinical importance and are maintained in a list in the product's Safety Review Plan.
- Tier 2 events: These are events that are not tier 1 but are "common." A MedDRA preferred term is defined as a tier 2 event if there is an incidence of at least 1% in any vaccine group.
- Tier 3 events: These are events that are neither tier 1 nor tier 2 events.

The Miettinen and Nurminen method[64] will be used to derive the 95% CI for the risk difference between *C difficile* vaccine and placebo for the above tier 1 and tier 2 events. The p-value from Miettinen and Nurminen method will also be provided for tier 1 events.

9.4.1. Reactogenicity

The proportion of subjects reporting local reactions at the injection site and systemic events on any day within the 7-day period after vaccination will be descriptively summarized by vaccine group.

Severities of local reactions and systemic events reported after each vaccination will also be descriptively summarized by vaccine group. Two (2)-sided 95% CIs based on the Clopper-Pearson method will be presented with the proportions.

9.5. Interim Analysis

Three (3) planned formal interim analyses will be performed by the independent statistical team (IST) to assess whether there is overwhelming efficacy or futility with respect to the end-of-study success criterion.

The first, second, and third interim analyses are planned after 30, 40, and 50 first primary CDI cases have occurred from 14 days after receipt of the third dose of investigational product, respectively. The data cutoff date will be the calendar date of the confirmed 30th, 40th, or 50th cases of first primary CDI occurring 14 days after the third dose by the central laboratory. Only the first primary efficacy endpoint will be examined. The statistical hypotheses testing (H_{03}) will be performed on interim data; H_{02} will be tested if H_{03} is rejected and a decision is made to proceed the final analysis

The early efficacy stopping boundary is derived on a GS design[65] (utilizing a single-binomial distribution, conditional on the total number of 66 cases, with 3 interim analyses at 45% [30 out of 66], 61% [40 out of 66], and 76% [50 out of 66] of information and a gamma[-2] alpha spending function).

The GS design from EAST (software package for clinical trial design) is based on normal approximation to binomial distribution, and EAST was used as a starting point to calculate GS design boundaries. Those were later converted into discrete boundaries (expressed as numbers of cases rather than proportions of cases coming from a vaccine group, given the total number of cases).

If there are 6 or fewer cases observed in the *C difficile* vaccine group out of a total of 30 first primary cases (point estimate of VE ≥75.0% and corresponding to a lower bound of 2-sided 98.8% exact CI >20%), the null hypothesis in H_{03} will be rejected, the *C difficile* vaccine will be deemed efficacious, and a full analysis of the data will be conducted.

If there are 9 or fewer cases observed in the *C difficile* vaccine group out of a total of 40 first primary cases in the second interim analysis (point estimate of VE ≥71.0% and corresponding to a lower bound of 2-sided 98.8% exact CI >20%), the null hypothesis in H_{03} will be rejected, the *C difficile* vaccine will be deemed efficacious, and a full analysis of the data may be conducted.

If there are 13 or fewer cases observed in the *C difficile* vaccine group out of a total of 50 first primary cases in the third interim analysis (point estimate of VE ≥64.9% and corresponding to a lower bound of 2-sided 98.2% exact CI >20%), the null hypothesis in H_{03} will be rejected, the *C difficile* vaccine will be deemed efficacious, and a full analysis of the data may be conducted.

Of note, using the lower bound of a 2-sided 98.8% CI corresponds to α=0.006 (one-sided) used for the first and second interim analyses; the lower bound of a 2-sided 98.2% CI corresponds to α=0.009 (one-sided) for the third interim analysis.

The probabilities of stopping the trial for success are 44.3% at interim analysis 1 (ie, observe ≤6 cases in the *C difficile* vaccine group), 15.0% at interim analysis 2 (ie, observe ≤9 cases in the *C difficile* vaccine group), and 17.1% at interim analysis 3 (ie, observe ≤13 cases in the *C difficile* vaccine group) if the true VE is 70%.

Study futility at each interim analysis will be evaluated based on the pre-identified case split (*C difficile* vaccine vs placebo) by analysis of conditional power (CP) approach. The conditional probability of study success at the final analysis with observed VE of at least 59.6% (19 out of 66 cases) is calculated based on an assumed case split in the *C difficile* vaccine group and the assumption that an adjusted true VE of 66.5% remains for the rest of the study. The case split for futility is 11 versus 19 for IA1 (corresponding to a CP cutoff of 50% at 30 cases), 13 versus 27 for IA2 (corresponding to a CP cutoff of 60% at 40 cases), and 16 versus 34 for IA3 (corresponding to a CP cutoff of 60% at 50 cases).

These futility boundaries may be subject to change to reflect subsequent program-related decisions by the Sponsor.

The probability of stopping the trial because of futility is 6.6% at interim analysis 1 (ie, observe ≥11 cases in the *C difficile* vaccine group), 5.8% at interim analysis 2 (ie, observe ≥13 cases in the *C difficile* vaccine group), and 2.0% at interim analysis 3 (ie, observe ≥16 cases in the *C difficile* vaccine group), if the true VE is 70%.

The overall probability of success is at least 84.8% if the true VE is 70%.

Additional futility assessments for the study might be carried out at the time of external data monitoring committee (E-DMC) meetings. Full details of the futility assessment will be provided in the SAP.

There is no impact on the overall type I error rate for futility analyses performed for the study.

Additional design operating characteristics (stopping boundary based on the number of cases observed in the *C difficile* vaccine group; the probabilities of stopping for efficacy and futility given assumed various VEs with a 1:1 randomization ratio) are listed in Table 7.

Table 7. Statistical Design Operating Characteristics

	Interim Analysis #1 (Total Cases = 30)		**Interim Analysis #2 (Total Cases = 40)**		**Interim Analysis #3 (Total Cases = 50)**		**Final Analysis (Total Cases = 66)**		
Vaccine Efficacy (%)	**Probability of Success (Cases in *C difficile* Vaccine Group ≤ 6)**	**Probability of Failure (Cases in *C difficile* Vaccine Group ≥ 11)**	**Probability of Success (Cases in *C difficile* Vaccine Group ≤ 9)**	**Probability of Failure (Cases in *C difficile* Vaccine Group ≥ 13)**	**Probability of Success (Cases in *C difficile* Vaccine Group ≤ 13)**	**Probability of Failure (Cases in *C difficile* Vaccine Group ≥ 16)**	**Probability of Success (Cases in *C difficile* Vaccine Group ≤ 20)**	**Probability of Failure (Cases in *C difficile* Vaccine Group ≥ 21)**	**Overall Probability of Success**
0	0.0007	0.9506	0.0002	0.0416	0.0003	0.0050	0.0002	0.0014	0.0014
20	0.0047	0.8512	0.0017	0.1066	0.0029	0.0211	0.0033	0.0085	0.0126
50	0.0838	0.4152	0.0397	0.2032	0.0721	0.0709	0.0739	0.0410	0.2696
60	0.2039	0.2146	0.0909	0.1490	0.1424	0.0557	0.1134	0.0300	0.5507
70	0.4427	0.0656	0.1496	0.0583	0.1711	0.0202	0.0845	0.0080	0.8479
80	0.7765	0.0067	0.1207	0.0063	0.0730	0.0015	0.0149	0.0003	0.9852

Note: Assumes 95.7% assay sensitivity and 99.1% assay specificity. Assumes equal follow-up times between vaccine groups.

Should the actual amount of information observed at interim analysis be more than the planned information capture for each interim analysis, the first targeted primary cases will be used for the analysis.

9.6. Data Monitoring Committee

This study will use an E-DMC.

The E-DMC will be responsible for ongoing monitoring of VE, futility, and safety of subjects in the study according to the charter. The E-DMC may review unblinded data during closed meeting sessions; however, the sponsor will remain blinded and will not be permitted access to the randomization assignments until the database is locked.

The E-DMC will review efficacy/futility and safety data at defined intervals as specified in the charter. The E-DMC may conduct additional meetings to review safety data at other time points during the study, at its discretion, or at the request of the sponsor. In addition, as determined by the sponsor clinician, the E-DMC may meet on an ad hoc basis to evaluate any SAEs related to vaccination, or related AEs that may jeopardize further subject participation, in order to determine that the study may be continued safely.

The E-DMC will work in association with the IST in order to conduct both the safety and futility/efficacy evaluations. The IST will include statistician(s) and programmer(s) who are independent of the sponsor and who have unrestricted access to the randomization assignments during the study. The IST will perform statistical analysis and prepare unblinded data and reports to provide to the E-DMC for assessment in accordance with the E-DMC charter. The IST will report the outcome of the planned futility checks (only whether the futility rule has been passed or not) to the sponsor and the E-DMC separately.

After each meeting, the E-DMC will make recommendations that may include the following: continue the study with or without modification, pause or stop vaccination for safety or other reasons, or pause or stop the study for efficacy or futility (assessed at an efficacy/futility meeting only).

The recommendations made by the E-DMC to alter the conduct of the study will be forwarded to Pfizer for final decision. Information on the current efficacy cases (numbers occurring in the *C difficile* vaccine and placebo groups) will not be disclosed until a decision is made to permanently stop the study. Pfizer will forward such decisions, which may include summaries of aggregate analyses of endpoint events and of safety data that are not endpoints, to regulatory authorities, as appropriate.

10. QUALITY CONTROL AND QUALITY ASSURANCE

Pfizer or its agent will conduct periodic monitoring visits during study conduct to ensure that the protocol and GCPs are being followed. The monitors may review source documents to confirm that the data recorded on CRFs are accurate. The investigator and institution will allow Pfizer monitors/auditors or its agents and appropriate regulatory authorities direct access to source documents to perform this verification. This verification may also occur after study completion.

During study conduct and/or after study completion, the investigator site may be subject to review by the IRB/EC, and/or to quality assurance audits performed by Pfizer, or companies working with or on behalf of Pfizer, and/or to inspection by appropriate regulatory authorities.

The investigator(s) will notify Pfizer or its agents immediately of any regulatory inspection notification in relation to the study. Furthermore, the investigator will cooperate with Pfizer or its agents to prepare the investigator site for the inspection and will allow Pfizer or its agent, whenever feasible, to be present during the inspection. The investigator site and investigator will promptly resolve any discrepancies that are identified between the study data and the subject's medical records. The investigator will promptly provide copies of the inspection findings to Pfizer or its agent. Before response submission to the regulatory authorities, the investigator will provide Pfizer or its agents with an opportunity to review and comment on responses to any such findings.

It is important that the investigator(s) and their relevant personnel are available during the monitoring visits and possible audits or inspections and that sufficient time is devoted to the process.

11. DATA HANDLING AND RECORD KEEPING

11.1. Case Report Forms/Electronic Data Record

As used in this protocol, the term CRF should be understood to refer to either a paper form or an electronic data record or both, depending on the data collection method used in this study.

A CRF is required and should be completed for each included subject. The completed original CRFs are the sole property of Pfizer and should not be made available in any form to third parties, except for authorized representatives of Pfizer or appropriate regulatory authorities, without written permission from Pfizer.

The investigator has ultimate responsibility for the collection and reporting of all clinical, safety, and laboratory data entered on the CRFs and any other data collection forms (source documents) and ensuring that they are accurate, authentic/original, attributable, complete, consistent, legible, timely (contemporaneous), enduring, and available when required. The CRFs must be signed by the investigator or by an authorized staff member to attest that the data contained on the CRFs are true. Any corrections to entries made in the CRFs or source documents must be dated, initialed, and explained (if necessary) and should not obscure the original entry.

In most cases, the source documents are the hospital or the physician subject chart. In these cases, data collected on the CRFs must match the data in those charts.

In some cases, the CRF may also serve as the source document. In these cases, a document should be available at the investigator site and at Pfizer that clearly identifies those data that will be recorded on the CRF, and for which the CRF will stand as the source document.

11.2. Record Retention

To enable evaluations and/or inspections/audits from regulatory authorities or Pfizer, the investigator agrees to keep records, including the identity of all participating subjects (sufficient information to link records, eg, CRFs and hospital records), all original signed ICDs, copies of all CRFs, safety reporting forms, source documents, and detailed records of treatment disposition, and adequate documentation of relevant correspondence (eg, letters, meeting minutes, and telephone call reports). The records should be retained by the investigator according to the ICH guidelines, according to local regulations, or as specified in the clinical study agreement (CSA), whichever is longer.

If the investigator becomes unable for any reason to continue to retain study records for the required period (eg, retirement, relocation), Pfizer should be prospectively notified. The study records must be transferred to a designee acceptable to Pfizer, such as another investigator, another institution, or an independent third party arranged by Pfizer.

Investigator records must be kept for a minimum of 15 years after completion or discontinuation of the study or for longer if required by applicable local regulations.

The investigator must obtain Pfizer's written permission before disposing of any records, even if retention requirements have been met.

12. ETHICS

12.1. Institutional Review Board/Ethics Committee

It is the responsibility of the investigator to have prospective approval of the study protocol, protocol amendments, ICDs, and other relevant documents, eg, recruitment advertisements, if applicable, from the IRB/EC. All correspondence with the IRB/EC should be retained in the investigator file. Copies of IRB/EC approvals should be forwarded to Pfizer.

The only circumstance in which an amendment may be initiated prior to IRB/EC approval is where the change is necessary to eliminate apparent immediate hazards to the subjects. In that event, the investigator must notify the IRB/EC and Pfizer in writing immediately after the implementation.

12.2. Ethical Conduct of the Study

The study will be conducted in accordance with the protocol, legal and regulatory requirements, and the general principles set forth in the International Ethical Guidelines for Biomedical Research Involving Human Subjects (Council for International Organizations of Medical Sciences 2002), ICH Guideline for Good Clinical Practice, and the Declaration of Helsinki.

12.3. Subject Information and Consent

All parties will ensure protection of subject personal data and will not include subject names or other identifiable data in any reports, publications, or other disclosures, except where required by law.

When study data are compiled for transfer to Pfizer and other authorized parties, subject names, addresses, and other identifiable data will be replaced by numerical codes based on a numbering system provided by Pfizer in order to de-identify study subjects. The investigator site will maintain a confidential list of subjects who participated in the study, linking each subject's numerical code to his or her actual identity. In case of data transfer, Pfizer will maintain high standards of confidentiality and protection of subjects' personal data consistent with applicable privacy laws.

The informed consent documents and any subject recruitment materials must be in compliance with ICH GCP, local regulatory requirements, and legal requirements, including applicable privacy laws.

The informed consent documents used during the informed consent process and any subject recruitment materials must be reviewed and approved by Pfizer, approved by the IRB/EC before use, and available for inspection.

The investigator must ensure that each study subject is fully informed about the nature and objectives of the study and possible risks associated with participation.

The investigator, or a person designated by the investigator, will obtain written informed consent from each subject before any study-specific activity is performed. The investigator will retain the original of each subject's signed consent document.

12.4. Reporting of Safety Issues and Serious Breaches of the Protocol or ICH GCP

In the event of any prohibition or restriction imposed (ie, clinical hold) by an applicable regulatory authority in any area of the world, or if the investigator is aware of any new information that might influence the evaluation of the benefits and risks of the investigational product, Pfizer should be informed immediately.

In addition, the investigator will inform Pfizer immediately of any urgent safety measures taken by the investigator to protect the study subjects against any immediate hazard, and of any serious breaches of this protocol or of ICH GCP that the investigator becomes aware of.

13. DEFINITION OF END OF TRIAL

13.1. End of Trial in a Member State

End of trial in a Member State of the European Union is defined as the time at which it is deemed that a sufficient number of subjects have been recruited and completed the study as stated in the regulatory application (ie, clinical trial application [CTA]) and ethics application in the Member State. Poor recruitment (recruiting less than the anticipated number in the CTA) by a Member State is not a reason for premature termination but is considered a normal conclusion to the study in that Member State.

13.2. End of Trial in All Other Participating Countries

End of trial in all other participating countries is defined as database lock.

14. SPONSOR DISCONTINUATION CRITERIA

Premature termination of this study may occur because of a regulatory authority decision, change in opinion of the IRB/EC, or investigational product safety problems, or at the discretion of Pfizer. In addition, Pfizer retains the right to discontinue development of *C difficile* vaccine at any time.

If a study is prematurely terminated, Pfizer will promptly notify the investigator. After notification, the investigator must contact all participating subjects and the hospital pharmacy (if applicable) within 28 days. As directed by Pfizer, all study materials must be collected and all CRFs completed to the greatest extent possible.

15. PUBLICATION OF STUDY RESULTS

15.1. Communication of Results by Pfizer

Pfizer fulfills its commitment to publicly disclose clinical trial results through posting the results of studies on www.clinicaltrials.gov (ClinicalTrials.gov), the European Clinical Trials Database (EudraCT), and/or www.pfizer.com, and other public registries in accordance with applicable local laws/regulations.

In all cases, study results are reported by Pfizer in an objective, accurate, balanced, and complete manner and are reported regardless of the outcome of the study or the country in which the study was conducted.

www.clinicaltrials.gov

Pfizer posts clinical trial US Basic Results on www.clinicaltrials.gov for Pfizer-sponsored interventional studies (conducted in patients) that evaluate the safety and/or efficacy of a Pfizer product, regardless of the geographical location in which the study is conducted. US Basic Results are submitted for posting within 1 year of the primary completion date (PCD) for studies in adult populations or within 6 months of the PCD for studies in pediatric populations.

PCD is defined as the date that the final subject was examined or received an intervention for the purposes of final collection of data for the primary outcome, whether the clinical study concluded according to the prespecified protocol or was terminated.

EudraCT

Pfizer posts European Union (EU) Basic Results on EudraCT for all Pfizer-sponsored interventional studies that are in scope of EU requirements. EU Basic Results are submitted for posting within 1 year of the PCD for studies in adult populations or within 6 months of the PCD for studies in pediatric populations.

www.pfizer.com

Pfizer posts Public Disclosure Synopses (clinical study report synopses in which any data that could be used to identify individual patients has been removed) on www.pfizer.com for Pfizer-sponsored interventional studies at the same time the US Basic Results document is posted to www.clinicaltrials.gov.

15.2. Publications by Investigators

Pfizer supports the exercise of academic freedom and has no objection to publication by the principal investigator (PI) of the results of the study based on information collected or generated by the PI, whether or not the results are favorable to the Pfizer product. However, to ensure against inadvertent disclosure of confidential information or unprotected inventions, the investigator will provide Pfizer an opportunity to review any proposed publication or other type of disclosure of the results of the study (collectively, "publication") before it is submitted or otherwise disclosed.

The investigator will provide any publication to Pfizer at least 30 days before it is submitted for publication or otherwise disclosed. If any patent action is required to protect intellectual property rights, the investigator agrees to delay the disclosure for a period not to exceed an additional 60 days.

The investigator will, on request, remove any previously undisclosed confidential information before disclosure, except for any study- or Pfizer product-related information necessary to the appropriate scientific presentation or understanding of the study results.

If the study is part of a multicenter study, the investigator agrees that the first publication is to be a joint publication covering all investigator sites, and that any subsequent publications by the PI will reference that primary publication. However, if a joint manuscript has not been submitted for publication within 12 months of completion or termination of the study at all participating sites, the investigator is free to publish separately, subject to the other requirements of this section.

For all publications relating to the study, the institution will comply with recognized ethical standards concerning publications and authorship, including Section II - "Ethical Considerations in the Conduct and Reporting of Research" of the Uniform Requirements for Manuscripts Submitted to Biomedical Journals, http://www.icmje.org/index.html#authorship, established by the International Committee of Medical Journal Editors.

Publication of study results is also provided for in the CSA between Pfizer and the institution. In this section entitled Publications by Investigators, the defined terms shall have the meanings given to them in the CSA.

If there is any conflict between the CSA and any attachments to it, the terms of the CSA control. If there is any conflict between this protocol and the CSA, this protocol will control as to any issue regarding treatment of study subjects, and the CSA will control as to all other issues.

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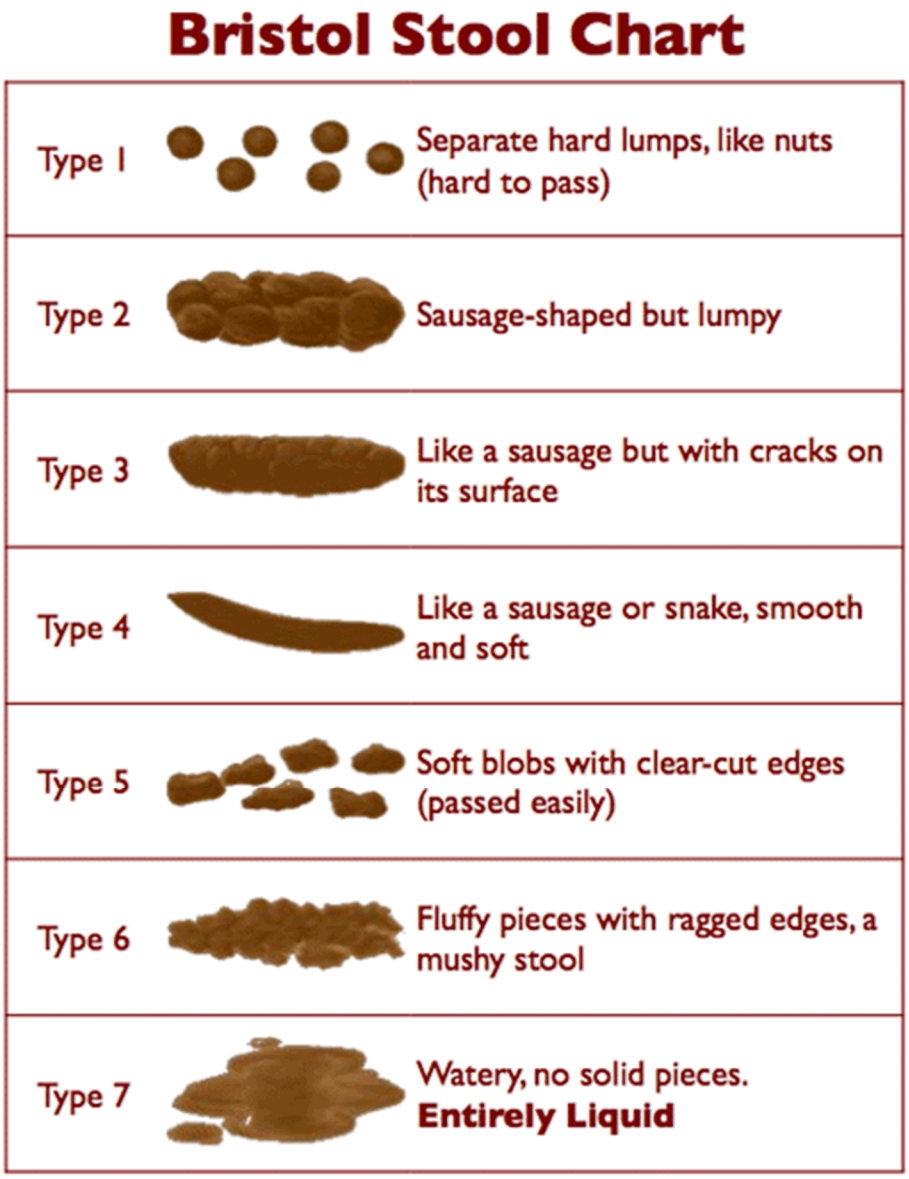
Appendix 1. Abbreviations

The following is a list of abbreviations that may be used in the protocol.

Abbreviation	Term
AE	adverse event
AlOH	aluminum hydroxide
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATLAS	**a**ge, **t**reatment with systemic antibiotics during *Clostridium difficile* infection therapy, **l**eukocyte count, serum **a**lbumin, and **s**erum creatinine
CBER	Center for Biologics Evaluation and Research
CCI	Charlson Comorbidity Index
CDI	*Clostridium difficile* infection
CE	Conformité Européenne
CI	confidence interval
CK	creatine kinase
CP	conditional power
CRA	clinical research associate
CRF	case report form
CSA	clinical study agreement
CT	clinical trial
CTA	clinical trial application
DILI	drug-induced liver injury
DU	dispensable unit
EC	ethics committee
e-diary	electronic diary
E-DMC	external data monitoring committee
EDP	exposure during pregnancy
ELISA	enzyme-linked immunosorbent assay
EU	European Union
EudraCT	European Clinical Trials Database
FDA	Food and Drug Administration (United States)
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
GGT	gamma-glutamyl transferase
GMFR	geometric mean fold rise
GS	group-sequential
HIV	human immunodeficiency virus
IB	investigator's brochure
ICD	informed consent document

Abbreviation	Term
ICH	International Conference on Harmonisation
ID	identification
IgG	immunoglobulin G
IND	investigational new drug application
INR	international normalized ratio
IP	investigational product
IRB	institutional review board
IRR	infection rate ratio
IRT	interactive response technology
IST	independent statistical team
IUD	intrauterine device
IWR	interactive Web-based response
LFT	liver function test
LL	lower limit
mAb	monoclonal antibody
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent-to-treat
N/A	not applicable
PCD	primary completion date
PCR	polymerase chain reaction
PFGE	pulsed-field gel electrophoresis
PI	principal investigator
PT	prothrombin time
SAE	serious adverse event
SAP	statistical analysis plan
SRM	study reference manual
SRSD	single reference safety document
TBili	total bilirubin
TcdA	*Clostridium difficile* toxin A
TcdB	*Clostridium difficile* toxin B
UL	upper limit
ULN	upper limit of normal
US	United States
VE	vaccine efficacy

Appendix 2. Bristol Stool Chart



Appendix 3. France Appendix

This appendix applies to study sites located in France.

1. GCP Training

Prior to enrollment of any subjects, the investigator and any subinvestigators will complete the Pfizer-provided Good Clinical Practice training course ("Pfizer GCP Training") or training deemed equivalent by Pfizer. Any investigators who later join the study will complete the Pfizer GCP Training or equivalent before performing study-related duties. For studies of applicable duration, the investigator and subinvestigators will complete Pfizer GCP Training or equivalent every three years during the term of the study, or more often if there are significant changes to the ICH GCP guidelines or course materials.

2. Investigational Product

No subjects or third-party payers will be charged for investigational product.

Appendix 4. Japan Appendix

The following supplementary text should be read in conjunction with the B5091007 protocol:

- An oral temperature of ≥37.5°C meets the definition of a current febrile illness in the criteria for temporarily delaying vaccine administration described in Section 4.3 for subjects enrolled in Japan.

- For the 2nd bulleted part of inclusion criterion 4, it is clarified that in Japan, subjects may be enrolled if, in the previous 12 months, they have had at least 2 emergency room visits for urgent or acute care.

- For the 3rd bulleted part of inclusion criterion 4, (under protocol amendments 1, 2, and 3) it is clarified that in Japan that "primary and/or secondary care visits" may have been undertaken in any type of healthcare facilities (primary, secondary [or tertiary]) for care directed by a physician. Note; Pharmacy and/or mental health visits do not count toward the 10 outpatient visits required under this criterion.

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Signed By:	Date(GMT)	Signing Capacity
PPD	28-Sep-2020 18:05:37	Final Approval