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

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

Clinical Trial Protocol – Amendment 6

Health Authority File Numbers:	BB-IND #: 7932 (US FDA) EudraCT: 2013-000775-32
WHO Universal Trial Number (UTN):	U1111-1127-7162
Trial Code:	H-030-014
Development Phase:	Phase III
Sponsor:	Sanofi Pasteur Inc. Discovery Drive, Swiftwater, PA 18370-0187, USA
Investigational Product:	*Clostridium difficile* Toxoid Vaccine
Form / Route:	Lyophilized (liquid for injection)/intramuscular (IM)
Indication For This Study:	Prevention of primary *C. difficile* infection (CDI)
Manufacturer:	Same as Sponsor
Coordinating Investigators:	As required by local regulations, there may be 1 Coordinating Investigator per country or 1 Coordinating Investigator per region. [names TBD]
Sponsor's Responsible Medical Officer:	[redacted], Sanofi Pasteur Inc.
Coordinating Clinical Trial Manager:	[redacted]
Trial Team:	See the "List of Investigators and Centers Involved in the Trial" document
Version and Date of the Protocol:	Version 7.0 dated 14 February 2017

This protocol Version 7.0 is the sixth amendment to the initial trial protocol Version 1.0, dated 09 November 2012.

[redacted]

Table of Contents

List of Tables

Synopsis

Company:	Sanofi Pasteur
Investigational Product:	*Clostridium difficile* Toxoid Vaccine
Active Substances:	*C. difficile* toxoids A & B

Title of the Trial:	Efficacy, Immunogenicity, and Safety Study of *Clostridium difficile* Vaccine in Subjects at Risk of *C. difficile* Infection (Cdiffense ™)
Development Phase:	Phase III
Coordinating Investigators:	According to local regulations, there may be 1 Coordinating Investigator per country or 1 Coordinating Investigator per region: names TBD
Trial Centers:	This will be a multi-national trial with approximately 350 trial centers. Investigators and sites will be listed in the "List of Investigators and Centers Involved in the Trial" document that will be provided.
Planned Trial Period:	3Q2013 to 2021 (estimated)
Trial Design and Methodology:	This is a randomized, observer-blind, placebo-controlled, multi-center, multi-national Phase III trial in 16,500 subjects. Adult subjects aged ≥ 50 years who are at risk for *C. difficile* infection (CDI) will be enrolled. Subjects will be enrolled in 1 of 2 risk strata across the treatment groups. Risk Stratum 1: • Has had at least 2 hospital stays, each lasting at least ≥ 24 hours, in the 12 months before enrollment and • Has received systemic (not topical) antibiotics in the 12 months before enrollment or Risk Stratum 2: • Is anticipated to have an in-patient hospitalization for a planned surgical procedure within 60 days of enrollment and the impending hospital stay is planned to be ≥ 72 hours (see also Inclusion Criteria) For stratification purpose, subjects who meet the criteria for both Risk Strata will be assigned to Risk Stratum 1. The study is designed as an event-driven group sequential protocol with (4 interim analyses at 50, 100, 135, 167 clinical endpoints (i.e. PCR-confirmed CDI episodes), and a final analysis when 250 clinical endpoints are reached. Subjects will be randomly assigned in a 2:1 ratio to receive either vaccine or placebo. Vaccine or placebo will be administered in a 3-dose schedule on Days 0, 7, and 30. At the time of group assignment, 1650 subjects (10% of total enrollment) will be randomly assigned to an immunogenicity subset; and 3300 subjects (20% of total enrollment) will be randomly assigned to a reactogenicity subset.

	All subjects will provide blood samples for immunogenicity assessment at Day 0 (pre-injection) and Day 60. Subjects in the immunogenicity subset from both study groups will also provide blood samples at Day 14, at Day 30 (pre-injection), and every 6 months through the planned follow-up periods (i.e., up to 3.0 years after the third injection). Solicited adverse reactions (ARs) will be collected for 6 days following each injection for subjects randomly assigned to the reactogenicity subset. In all subjects, unsolicited adverse events (AEs) will be collected from Day 0 to Day 60. All serious adverse events (SAEs) will be collected through the end of the surveillance period. All subjects will be actively followed for efficacy throughout the follow-up period, which may extend for up to 3 years after the last injection. Analyses of trial futility (non-efficacy) will be performed at the first 2 interim analyses. The study may be stopped if either of those analyses provides robust and compelling evidence that meaningful levels of vaccine efficacy (VE) will not be demonstrated. Minimal case splits (Vaccine [V]:Placebo [P]) and estimated VE that will result in declaration of futility are 33:17 (3% VE) and 62:38 (18% VE), at the first and second interim analysis, respectively. Analyses of efficacy will be performed at the second, third, and fourth interim analyses, as well as, the final analysis, with the total one-sided type 1 error rate at 0.025. The nominal significance levels for the 3 efficacy interim analyses are 0.01, 0.0035, and 0.0032, respectively, as determined by the corresponding proportion of information assessed at each interim analysis (number of new cases at each interim / 250 * 0.025). The remaining alpha (0.0083) after the interim analyses will be used at the final analysis. Based on trial simulations, if the true VE is 60%, there is an estimated 45% probability to reject the primary null hypothesis at the second interim (first interim for efficacy).
Primary Objective:	To assess the efficacy of the *C. difficile* vaccine in preventing the onset of symptomatic primary CDI confirmed by polymerase chain reaction (PCR) in adult subjects aged ≥ 50 years who are at risk for CDI and have received at least 1 injection.

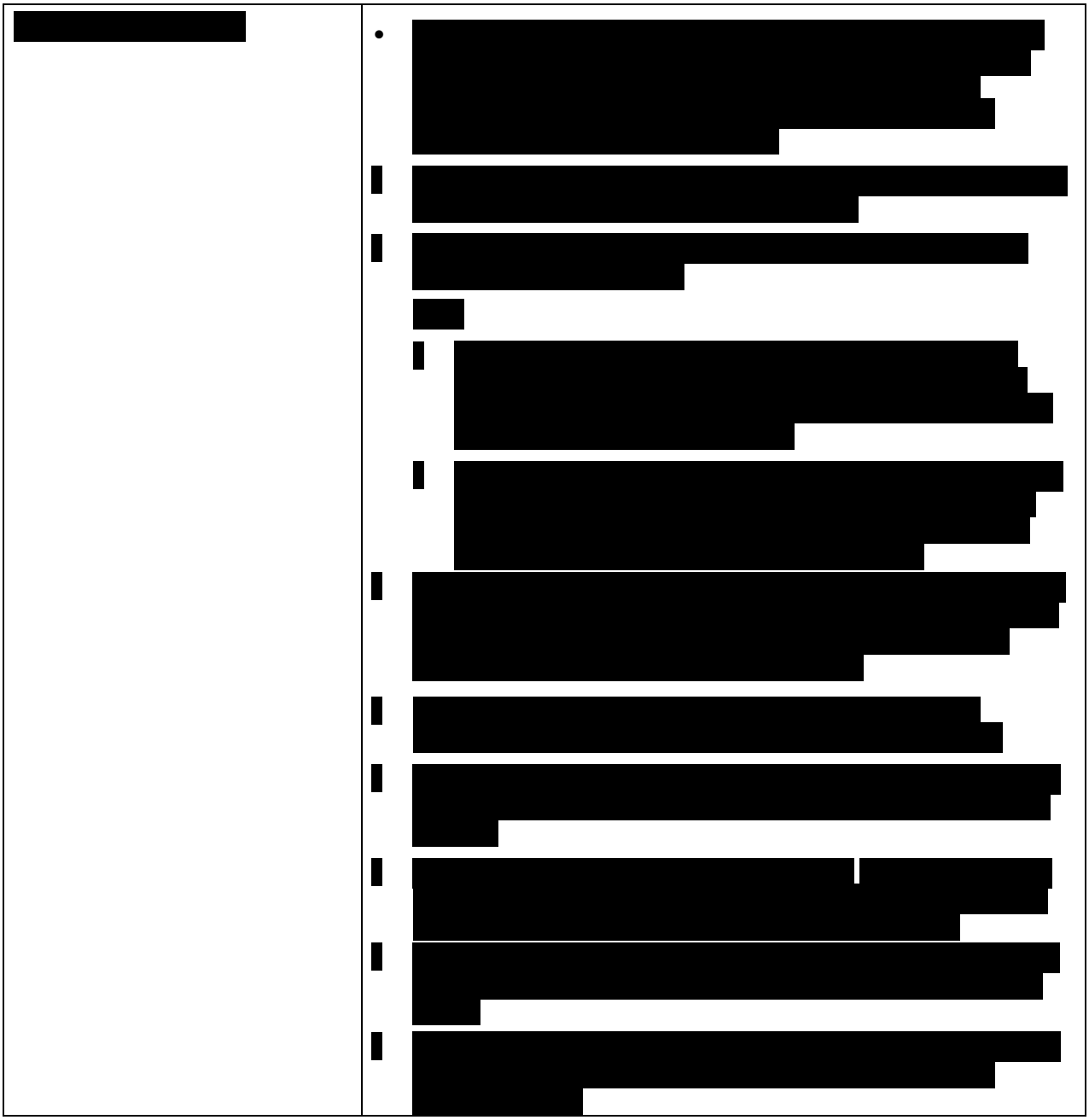
Primary Endpoint:	Symptomatic PCR-confirmed primary CDI cases, defined as:
	• Presence of both of the following clinical symptoms:
	  • ≥ 3 loose stools in ≤ 24-hours
	  • loose stools lasting ≥ 24 hours
	  Notes:
	  - Timing of 24-hour period will start from the first episode of loose stools
	  - Loose stool is defined as type 6 (fluffy pieces with ragged edges, a mushy stool) or type 7 (watery, no solid pieces; entirely liquid) according to the Bristol Stool Chart.
	and
	• Stool sample positive for *C. difficile* by PCR
	  Note: Only results from the PCR testing performed by the central laboratory will be used for analyses.
	or
	• Confirmatory test of pseudomembranous colitis diagnosed through colonoscopy, and, if available, provision of a stool sample for PCR-testing

Secondary Objectives:	<u>Efficacy:</u>
	• To assess prevention of symptomatic PCR-confirmed primary CDI cases after 3 injections administered at 0, 7, and 30 days
	• To assess prevention of symptomatic PCR-confirmed primary CDI cases after completion of at least 2 injections
	• To assess durability of prevention of symptomatic PCR-confirmed primary CDI cases up to 3.0 years after the third injection
	• To assess prevention of severe primary CDI cases in subjects with PCR-confirmed primary CDI
	• To assess the effect of the vaccine on reduction of loose stool frequency in subjects who are symptomatic primary PCR-confirmed CDI cases
	• To assess the effect of the vaccine on reduction of CDI episode/illness duration in subjects who are symptomatic primary PCR-confirmed CDI cases
	<u>Immunogenicity</u>:
	• To describe the immunogenicity to toxin A and toxin B:
	  • in the subset (1650 out of 16,500) of subjects and in subjects with CDI (250) at Day 0 and Day 60 (± 14 days)
	  and
	  • in the subset (1650 out of 16,500) of subjects at Day 14 (+3 days), Day 30 (-3 days to +7 days), and every 6 months up to 3.0 years (± 14 days) after the third injection

	<u>Safety</u>: • To describe the safety profile of all subjects who receive at least 1 injection

Secondary Endpoints:	Efficacy: • The number of symptomatic PCR-confirmed primary CDI cases after 3 injections (the per-protocol [PP] population) • The number of symptomatic PCR-confirmed primary CDI cases after at least 2 injections • The number of symptomatic PCR-confirmed primary CDI cases after 3 injections as a function of time since enrollment and within 3.0 years after the third injection • The number of severe PCR-confirmed primary CDI cases. A severe CDI case is defined when a subject has 1 or more of the following: fever ≥ 38.5°C, white blood cell count ≥ 15,000 cells/mm^3 (if available), ileus, pseudomembranous colitis, serum albumin <3 g/dl, abdominal distension, abdominal tenderness, or admission to the intensive care unit within 7 days of CDI diagnosis. • The maximum number of loose stools per day associated with a symptomatic PCR-confirmed primary CDI case • The CDI episode/illness duration associated with a symptomatic PCR-confirmed primary CDI case. Duration is calculated as (clinical cure date – clinical case date + 1) Immunogenicity: • Serum antibody concentrations against toxins A and B, measured by enzyme-linked immunosorbent assays (ELISA) • Serum antibody titers against toxins A and B, measured by toxin neutralization assay (TNA) • Exploratory assays may be performed Safety: 1) Occurrence, nature (Medical Dictionary for Regulatory Activities [MedDRA] preferred term), duration, intensity, and relationship to vaccination of any unsolicited systemic adverse events (AEs) reported in the 30 minutes after vaccination 2) Occurrence, time to onset, number of days of occurrence, intensity, action taken, and whether the reaction led to early termination from the study, of solicited (prelisted in the subject's diary card and eCRF) injection site reactions occurring up to 6 days after vaccination in subjects in the reactogenicity subset 3) Occurrence, time to onset, number of days of occurrence, intensity, action taken, and whether the reaction led to early termination from the study, of solicited (prelisted in the subject's diary card and eCRF) systemic reactions occurring up to 6 days after vaccination in subjects in the reactogenicity subset

	4) Occurrence, nature (MedDRA preferred term), time to onset, duration, intensity, action taken, relationship to vaccination (for systemic AEs only), and whether the event led to early termination from the study, of unsolicited AEs up to 30 days after vaccination 5) Occurrence, nature (MedDRA preferred term), time to onset, duration, seriousness criteria, relationship to vaccination, outcome, and whether the SAE led to early termination from the study, of SAEs through 6 months after the last injection 6) Occurrence, nature (MedDRA preferred term), time to onset, duration, seriousness criteria, outcome, and whether the SAE led to early termination from the study, of SAEs considered related to vaccination and/or study procedures 6 months after the last injection through the end of the follow-up period
[redacted]	[redacted]



Planned Sample Size:	A total of 16,500 subjects are planned to be enrolled: *C. difficile* Vaccine Group: n = 11,000 Placebo Group : n = 5500 Stratification will be based on the 2 major inclusion strata and will be defined as follows. Risk Stratum 1: • Has had at least 2 hospital stays, each lasting at least ≥ 24 hours, in the 12 months before enrollment and • Has received systemic (not topical) antibiotics in the 12 months before enrollment or Risk Stratum 2: • Is anticipated to have an in-patient hospitalization for a planned surgical procedure within 60 days of enrollment and the impending hospital stay is planned to be ≥ 72 hours (see also Inclusion Criteria). This stratification by region and risk stratum and fixed block size randomization will ensure the planned 2:1 balance between vaccine and placebo groups. The relative numbers of subjects enrolled into Risk Stratum 1 and Risk Stratum 2 will be monitored continuously during the trial. If the fraction enrolled to either stratum drops below 30%, then the study team will review the operational approaches to recruitment and recommend changes to the approach that will increase the relative rate of enrollment to the smaller group.
Schedule of Study Procedures:	Vaccination All subjects will receive 1 injection of either *C. difficile* vaccine or placebo at Days 0, 7, and 30. Blood sampling All subjects will provide a pre-vaccination blood sample at Day 0 and at Day 60. An immunogenicity subset (1650 subjects out of 16,500 subjects enrolled) will provide additional blood samples at Day 14, pre-vaccination on Day 30, and every 6 months during the follow-up period. Collection of safety data For subjects in the reactogenicity subset (3300 subjects out of 16,500 subjects enrolled), solicited reactions will be recorded for 6 days after each injection. All subjects will record information about unsolicited AEs through 30 days after the last injection. All SAEs (related and unrelated) will be collected in all subjects from Day 0 through the end of the follow-up period.

	Surveillance for CDI throughout the study and stool collection All subjects will be followed actively for efficacy (the primary occurrence or recurrence of a CDI) from Day 0 through the follow-up period for occurrence of repeated episodes of loose stools and provision of a stool sample. Some subjects may be followed for a shorter period of time if the trial is completed and the subject has not reached their maximum follow-up period. During the follow-up period, for those subjects who experience repeated episodes of loose stools, the third (or later) loose stool will be collected in the collection container provided. • A repeat stool sample will not be collected if a subject has already been diagnosed locally as a clinical case and the site has already frozen the stool sample for testing at the central laboratory. • A repeat stool sample will be collected for recurrence if the subject experiences episodes of loose stools ≥ 14 days following a clinical cure (which is defined as ≤ 2 loose stools in 24 hours for 2 consecutive days following completion of 10 days of standard of care treatment for the initial CDI episode). Recurrence is defined as the development of a new episode of diarrhea (≥ 3 loose stools in ≤ 24 hours and loose stools lasting ≥ 24 hours, and a PCR-confirmed CDI-positive stool sample). *Active CDI Surveillance:* There will be a weekly contact (telephone call, clinic visit, or home visit) for the 4 weeks immediately after enrollment in the study to remind subjects to report repeated episodes of loose stools and provide stool samples, if applicable. Following the first 4 weeks, subjects will be contacted every 2 weeks for the duration of the follow-up period. The method of contact may vary (by telephone, text message, email, and/or home visit); however, there must be subject contact via telephone or home visit once a month. If a subject is hospitalized or is admitted to a long-term care or rehabilitation facility, the subject will be contacted weekly until discharge or for a maximum of 3 months to monitor the subject for episodes of loose stools for which a stool sample will be obtained and provided to the site. After discharge from the hospital, long-term care or rehabilitation facility, the subject will be contacted weekly for 4 weeks, then every 2 weeks thereafter for the remainder of the follow-up period (as described above). For subjects in the immunogenicity subset, there will be a home or clinic visit every 6 months during the follow up period for collection of a blood sample. Details of the surveillance plan will be provided in the Operating Guidelines. [redacted]

Duration of Participation in the Trial:	Subjects may have a total duration of up to 3.0 years after Day 30.
Investigational Product: ***Form:*** ***Composition:***	*C. difficile* Toxoid Vaccine Lyophilized/liquid for injection [redacted]
Route: ***Batch Number:***	Intramuscular (IM) TBD
Control Product: ***Form:*** ***Composition:*** ***Route:*** ***Batch Number:***	Placebo Liquid 0.9% normal saline; 0.5 mL dose IM TBD

Inclusion Criteria:	An individual must fulfill all of the following criteria in order to be eligible for trial enrollment:
	1) Aged ≥ 50 years on the day of inclusion
	2) Informed consent form has been signed and dated
	3) Able to attend all scheduled visits and to comply with all trial procedures
	4) Covered by health insurance (only valid for specific countries)
	5) Must fulfill at least 1 of the following criteria*
	Risk Stratum 1:
	• Has had at least 2 hospital stays, each lasting at least ≥ 24 hours, in the 12 months before enrollment and
	• Has received systemic (not topical) antibiotics in the 12 months before enrollment
	or
	Risk Stratum 2:
	• Is anticipated to have an in-patient hospitalization for a planned surgical procedure within 60 days of enrollment. The impending hospital stay is planned to be ≥ 72 hours for a surgery involving 1 of the following:
	  • Kidney/bladder/urinary system
	  • Musculoskeletal system
	  • Respiratory system
	  • Circulatory system
	  • Central nervous system
	* If an individual is either an in-patient in the hospital or had a previous hospitalization, the enrollment date must be at least 30 days from hospital discharge.

Exclusion Criteria:	An individual fulfilling any of the following criteria is to be excluded from trial enrollment: 1) Subject is pregnant, or lactating, or of childbearing potential (to be considered of non-childbearing potential, a female must be post-menopausal for at least 1 year, surgically sterile, or using an effective method of contraception or abstinence from at least 4 weeks prior to the first vaccination and until at least 4 weeks after the last vaccination) 2) Participation in the 4 weeks preceding the first trial vaccination or participation during the present trial period in another clinical trial investigating a vaccine, drug, medical device, or medical procedure 3) Receipt of any vaccine in the 4 weeks preceding the first trial vaccination except for influenza (seasonal or pandemic) and pneumococcal vaccines. This exception includes monovalent pandemic influenza vaccines and multivalent influenza vaccines. 4) Previous vaccination against *C. difficile* with either the trial vaccine, another vaccine, or monoclonal antibodies 5) Diarrhea on day of enrollment 6) Self-reported current or prior CDI episode 7) Anticipated or current receipt of kidney dialysis treatment 8) History of gastrointestinal surgery for gastrointestinal malignancy (*Note:* Colonoscopy, polypectomy, and appendectomy are not exclusion criteria.) 9) History of inflammatory bowel disease, irritable bowel syndrome (must include diarrhea as a symptom), colostomy, or small or large intestine bowel surgery where resection was performed 10) Receiving enteral feeding (e.g., nasogastric tube, gastrostomy, or jejunostomy tube feeding) 11) Known or suspected congenital or acquired immunodeficiency; or receipt of immunosuppressive therapy, such as anti-cancer chemotherapy or radiation therapy, within the preceding 6 months; or long-term systemic corticosteroid therapy (prednisone or equivalent for more than 2 consecutive weeks within the past 3 months) 12) Known systemic hypersensitivity to any of the vaccine components, or history of a life-threatening reaction to the vaccine used in the trial or to a vaccine containing any of the same substances 13) Self-reported thrombocytopenia, contraindicating IM vaccination 14) Deprived of freedom by an administrative or court order, or in an emergency setting, or hospitalized involuntarily 15) Current alcohol abuse or drug addiction that might interfere with the ability to comply with trial procedures in the opinion of the Investigator 16) Chronic illness that, in the opinion of the investigator, is at a stage where it might interfere with trial conduct or completion 17) Moderate or severe acute illness/infection (according to investigator judgment) on the day of vaccination or febrile illness (temperature ≥ 38.0°C [≥ 100.4°F]). A prospective subject should not be included in the study until the condition has resolved or the febrile event has subsided.

	18) Identified as an Investigator or employee of the Investigator or study center with direct involvement in the proposed study, or identified as an immediate family member (i.e., parent, spouse, natural or adopted child) of the Investigator or employee with direct involvement in the proposed study
Statistical Methods:	***Primary statistical hypothesis*** The primary statistical null hypothesis is that there is no greater than a 15% reduction* in the risk of PCR-confirmed cases of CDI among the subjects receiving the vaccine relative to subjects receiving placebo. Formally the trial will test: H_0: Vaccine efficacy (VE) ≤ 15% *vs.* H_A: VE > 15% where VE=1-R_V/R_P and R_V and R_P are the rates of having a CDI endpoint during follow-up in the vaccinated and placebo groups, respectively. **Note*: There are small imperfections in the sensitivity and specificity of PCR that results in an attenuation of VE from that which would be realized under a perfect endpoint assay. We estimate that an observed 15% in PCR-confirmed cases of CDI corresponds to an estimated true VE of 20%; an observed VE of 46% corresponds to an estimated true VE of 60%. ***Statistical method of the primary analysis*** This is an event-driven, group sequential study design. There will be 4 interim analyses at 50, 100, 135, 167 clinical endpoints (i.e., PCR-confirmed CDI episodes), and a final analysis at 250 clinical endpoints. Futility testing will be performed at each of the first 2 interim analyses. Efficacy testing will be performed at the second, third, and fourth interim analyses, and at the final analysis, with the total one-sided type 1 error rate at 0.025. These tests will be based on computing point and interval estimates of VE using standard Poisson-based methods for estimating event rates and assessing whether the resulting confidence intervals (CIs) cover the null hypothesis value for VE. The nominal significance levels for the 3 efficacy interim analyses are 0.01, 0.0035, and 0.0032, respectively, as determined by the corresponding proportion of information assessed at each interim analysis (number of new cases at each interim / 250 * 0.025). The remaining alpha (0.0083) after the interim analysis will be used at the final analysis. The primary null hypothesis will be rejected if the CI computed at the nominal sizes describe above fail to cover the null VE value of 15%. The analyses will be performed on the modified intent-to-treat (MITT) population. We note that if interim analyses are performed based on total numbers of cases that are not precisely the same as the aforementioned target milestones, the nominal significance levels at which the interim analyses will be performed and those of the following tests will be adjusted accordingly. The tests for futility (non-efficacy) will be conducted as tests of the null hypothesis (futility) that VE is no greater than 46%, which corresponds to an estimated true VE of 60%. Formally, the following hypothesis will be used: H_0: VE ≥ 46% *vs.* H_A: VE < 46% where VE=1-R_V/R_P and R_V and R_P are the true rates of having a CDI endpoint during the follow-up period in the vaccine and placebo groups, respectively.

	The study will be stopped for futility (non-efficacy) at either of the 2 interim analyses if the upper limit of the 2-sided 95% CI for VE, calculated using standard Poisson-based estimates of relative endpoint rates amongst the vaccine and placebo groups, is less than the alternative hypothesis value for VE of 46%. The trial will be monitored by an Independent Data Monitoring Committee (IDMC) who will be the only group with access to the unblinded analyses at the 4 interim analyses. At each of the interim analyses, the IDMC will review the unblinded data and advise to either continue the trial as planned or to stop the trial (for efficacy or for futility) using the aforementioned statistical testing and operational criteria as primary guidance for their recommendation. In addition to the statistical guidelines for early stopping with a finding of efficacy, a recommendation of early stopping for efficacy by the IDMC will also require a minimum of a 12-month median duration. In case of an early stopping, each subject will be followed for safety for at least 6 months after the last injection. Under the trial design assumptions, at least a 12-month median duration of follow-up is expected to be obtained at the time of the fourth interim analysis (i.e., the third interim analysis for efficacy). However, if the projected duration of follow-up falls somewhat short of the 12-month requirement, the timing of that interim analysis will be delayed for a median of 12 months of follow up for all subjects is achieved in order to ensure that this operational criterion is met before the formal statistical test for efficacy. ***Statistical methods of the secondary analyses*** The same statistical methods used in the analyses of primary objectives will be used for those of the secondary objectives 1 through 4. However, the size of the associated tests and CIs computed for secondary analyses will not be formally adjusted for multiplicity because these analyses will be performed only once at the conclusion of the trial. 1) Efficacy to prevent CDI endpoints in the PP: the lower limit of the 2-sided 95% CI of the observed VE in the PP will be computed and compared to 15%. 2) Efficacy to prevent CDI endpoints in the subjects receiving 2 or more doses of the vaccine: the lower limit of the 2-sided 95% CI of the observed VE among the subset of subjects receiving 2 or more doses of the vaccine will be computed and compared to 15%. 3) Durability of the VE: a time-dependent model for the relative risk of PCR-confirmed CDI endpoints will be fit to the trial data to assess trends in VE as a function of time since enrollment. This model will be formally compared with the standard constant relative risk model to test (at 2-sided 0.05 level) whether any observed departures from constancy are statistically significant. 4) Efficacy to prevent severe CDI cases: relative rates of severe CDI will be computed for both the MITT and PP populations and, from these relative rates, the lower limit of the 2-sided 95% CI for VE will be computed and compared to 0%.

5) The frequency of subjects with the maximum loose stools per day in the vaccine and those in the placebo group during the CDI episode will be categorized into 3 categories; 3-5, 6-10 and >10 loose stools. The equality of the proportions of subjects in these 3 categories in the vaccine group and those in the placebo group will be tested using Fisher exact test using a 2-sided alpha of 0.05. If the test is significant, the assumption of equality of the effect of the two vaccination groups will be rejected.

6) The duration of each CDI episode/illness in the vaccine and the placebo groups will be collected and their distributions will be compared using the Log Rank Test using a one sided alpha of 0.05. If there is a significant reduction in CDI episode/illness duration in the vaccine group, the null hypothesis will be rejected.

- [redacted]

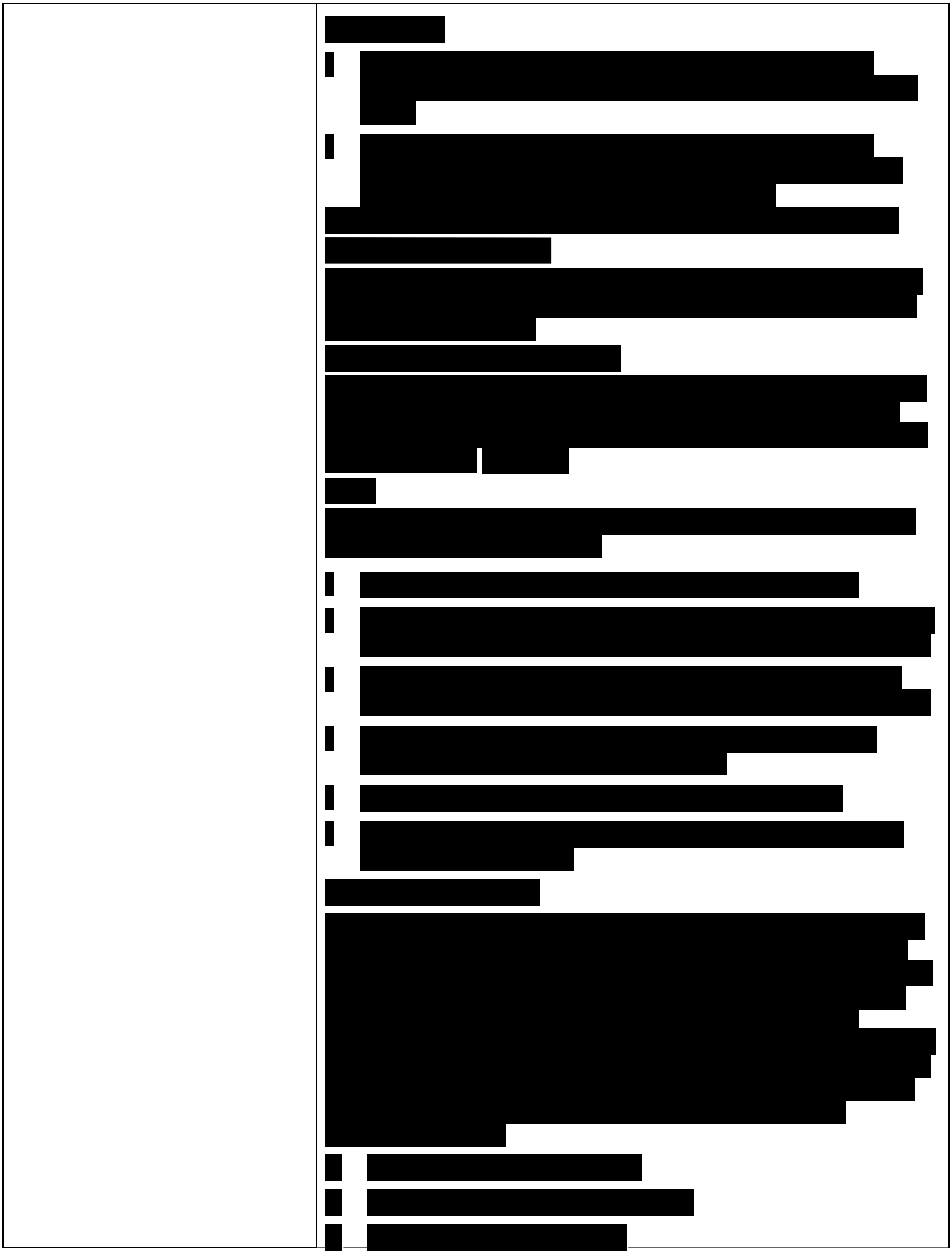


Table of Study Procedures for the Main Population:

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

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

**Note:* There is a separate Table of Study Procedures for subjects in the immunogenicity subset (N=1650)

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

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

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

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

‡ Blood sample collected pre-injection.

§ Diary cards and memory aid:

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Table of Study Procedures for the Immunogenicity Subset:

(N=1650 out of a total of 16,500)

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

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

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

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

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

[‡] Blood sample collected pre-injection.

[§] Diary cards and memory aid:

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

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

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

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

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

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

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

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

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

visit once a month.

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

For subjects in the immunogenicity subset, there will be a home or clinic visit every 6 months during the follow-up period after the last injection for collection of a blood sample.

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

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

List of Abbreviations

AE	adverse event
AR	adverse reaction
BL	blood sample
C	Celsius
CDI	*C. difficile* infection
CDM	Clinical Data Management
CI	confidence interval
CRA	Clinical Research Associate
CRO	Contract Research Organization
(R)CTM	(Regional) Clinical Trial Manager
DC	diary card
eCRF	electronic case report form
EDC	electronic data capture
ELISA	enzyme-linked immunosorbent assay
EIP	Emerging Infection Program
EU	ELISA unit
F	Fahrenheit
FAS	full analysis set
GCP	Good Clinical Practice
GM	geometric mean
GMC	geometric mean concentration
GMT	geometric mean titer
GPV	Global Pharmacovigilance Department
IATA	International Air Transport Association
ICF	informed consent form
ICH	International Conference on Harmonization
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
IgG	immunoglobulin G
IND	Investigational New Drug (application)
IRB	Institutional Review Board
IVIG	immunoglobulin G administered intravenously
IVRS	interactive voice response system
IWRS	interactive web response system
LLT	lowest level term

MA	memory aid
MA-LS	memory aid for loose stools
MedDRA	Medical Dictionary for Regulatory Activities
MITT	modified intent-to-treat analysis set
mL	milliliter
mM	millimolar
NSAID	non-steroidal anti-inflammatory drug
P	Placebo
PCR	polymerase chain reaction
PP	per-protocol analysis set
PSO	Product Safety Officer
QA	quality assurance
R&D	Research and Development
REA	restriction endonuclease analysis
RMO	Responsible Medical Officer
SAE	serious adverse event
SafAS	safety analysis set
SAP	Statistical Analysis Plan
SOC	system organ class
TMF	Trial Master File
TNA	toxin neutralization assay
UAR	unexpected adverse reaction
UTN	universal trial number
V	Vaccine
VAS	Visual Analogue Scale
VE	vaccine efficacy
WBC	white blood cell count
WHO	World Health Organization

1 Introduction

1.1 Background

Sanofi Pasteur is developing a vaccine against primary symptomatic *Clostridium difficile* infection (CDI) in at-risk individuals. At-risk individuals are defined as adults requiring frequent and or prolonged antibiotic use and exposure to the healthcare environment, which may include hospitalization and/or long-term care, nursing home, or rehabilitation admission. Given the increasing reports of this infection, treatment failures, and high rates of recurrence (1), CDI is an important target for prevention through vaccination worldwide and will fulfill an unmet medical need.

C. difficile is a ubiquitous, gram-positive, spore-forming anaerobic bacterium that is the most common cause of infectious nosocomial diarrhea, and is responsible for up to 30% of all diarrhea cases among hospitalized patients (2). The primary reservoirs of *C. difficile* within hospitals and long-term care facilities include colonized or infected patients and contaminated environments and surfaces (2) (3). Once colonized, patients with *C. difficile* shed spores in their feces and can transmit the spores to the environment, to include other patients and health care workers. The incidence is also increasing among persons living in the community with recent healthcare contact (4).

Acquisition of *C. difficile* can cause manifestations ranging from asymptomatic colonization to mild self-limited diarrhea, abdominal pain, severe diarrhea, pseudomembranous colitis, colonic perforation, and death. Systemic complications include ascites, pleural effusion, cardiopulmonary arrest, and multiple organ failure (5). The symptoms of *C. difficile* infection are mediated by the production of two large protein toxins: toxin A (308-kDa) and toxin B (270-kDa). Most toxigenic strains of *C. difficile* produce both toxins; a minority of them produce toxin B only. The relative importance of each toxin in causing disease in humans is not fully elucidated; however, strains producing toxin B only can cause the full spectrum of disease manifestations. Non-toxigenic *C. difficile* strains do not cause disease symptoms and therefore are non-pathogenic (6) (7). Toxigenic strains of *C. difficile* induce clinical symptoms by causing acute inflammation of the intestinal mucosa characterized by an abundant neutrophil infiltrate, epithelial damage, increased permeability (6) (7) (8), and transudation of serum proteins (including immunoglobulin G [IgG]) in the mucosa (9).

1.1.1 CDI Risk Factors

CDI risk factors are well documented in the literature and can be classified into 2 categories: (a) the endogenous risk factors, which are linked to the individual characteristics of the patients, like age and co-morbidities, and (b) the exogenous risk factors, quantifying the exposure to the risk either through the CDI pressure (frequency of the contact with the health care system, i.e. potentially to the *C. difficile* spores) or through antibiotic use. Most of the risk factors are inter-related (co-morbidities and high age lead to higher hospitalization and antibiotic use), making it challenging to evaluate their marginal effects. The combination of these different risk factors permits definition of at-risk populations.

There is a lack of standardized reporting mechanisms of CDI in most countries worldwide. However, the CDI incidence in hospital is documented by surveillance data in several countries. CDI incidence in the general hospital population is relatively consistent between US, Canada, and Europe within a range between 4 to 9 per 10,000 patients-days (10) (11) (12). Data from Latin America and South-East Asia are limited.

In long-term care facilities and nursing homes, little to no CDI surveillance data exists but rates of disease and/or colonization are potentially high given the concentration of known risk factors for CDI (length of health care stay, elderly patients, exposure to antimicrobials). In the community, the incidence of CDI was estimated between 7 to 25 CDI per 100,000 persons-years (13) (14). Recent data from the Emerging Infection Program (EIP) in the US reported that of all CDIs, 94% of all CDI cases are related to health-care exposures. Among health-care–associated CDIs, 75% have their onset outside of hospitals and 52% of the CDIs treated in hospitals are present on admission, representing an important source for intra-hospital transmission (15).

Overall, the incidence and the severity of CDI cases have been increasing in recent years and remain underestimated (16). The increased use of the polymerase chain reaction (PCR) testing for CDI early detection worldwide to replace the low sensitive enzyme immunoassay (EIA) currently used routinely in most countries will also probably increase the reported number of CDI.

The increase in severity is associated with the recent identification of a hypervirulent strain BI/NAP1/027 10 (17).

Accurate mortality rates attributable to CDI are difficult to assess due to the under-reporting of CDI and due to the commonly observed high level of co-morbidities among CDI patients leading to difficulty in determining level of attribution. A recent paper of Karas *et al.* reviewed the published literature on CDI attributable mortality including 27 European and non-European studies from 1980 to 2010 reflecting 10,975 CDI cases (18). Overall mortality attributable to CDI was approximately 6% within 3 months of diagnosis, rising to 13 to 15% in older patients. After the year 2000, the rate was 8% for the general population.

A separate document will be available that details the burden of disease attributable to *C. difficile* in different regions of the world with a focus on candidate countries for the Phase III efficacy study.

1.1.2 Current treatment for CDI

Until recently, therapeutic options for the treatment of CDI have been limited to vancomycin, metronidazole, and a few other antibacterials, such as nitazoxanide, rifaximin, and bacitracin. In May 2011, the US FDA approved fidaxomicin for the treatment of CDI in adults. The available evidence suggests that for patients with non-severe disease, fidaxomicin, metronidazole, and vancomycin all provide effective treatment. Recurrence is common with all agents but develops less frequently with fidaxomicin when compared to vancomycin (19). Several other CDI targeted antimicrobial and non-antimicrobial agents (oral administration of non-toxigenic CDI and fecal microbiota transplantation) are in various stages of development as current therapeutics are suboptimal secondary to the high incidence of relapsing disease and the potential for emergence of drug-resistance in non-clostridial bacteria.

1.1.3 Immune-based approach supporting CDI prevention

There is strong evidence that these host immune responses to *C. difficile* toxins A and B have a substantial role in determining the clinical outcome of *C. difficile* infection. Serum anti-toxin IgG antibody levels have been found to play an important role in determining the outcome of colonization and protection against a first episode of CDI. Patients who are asymptomatically colonized with *C. difficile* have higher serum anti-toxin A IgG levels, measured by enzyme-linked immunosorbent assay (ELISA), than colonized patients who develop diarrhea (20). This suggests that high anti-toxin A IgG levels at the time of colonization protect against CDI. Once colonization has progressed to CDI, IgG antibody responses to *C. difficile* toxins A, B have been shown to be higher in patients who experience a single episode of CDI compared to patients with recurrent CDI (21). An effective serum IgG antibody response to *C. difficile* toxins is important in influencing the outcome of colonization and provides the basis for the development of antibody-mediated therapeutics and vaccines for *C. difficile* (22).

An early formulation of the Sponsor's *C. difficile* Toxoid Vaccine (in H-030-005 study – 6.25 µg, 25 µg, and 100 µg with and without aluminum containing adjuvant with schedule on Days 1, 8, 30, and 60) was safely tolerated in healthy adults after IM administration and was shown to induce high serum anti-toxin IgG antibody levels that were as good or better than those in sera from colonized individuals who did not exhibit CDI symptoms (23) (24). In vaccinees, the concentrations of anti-toxin IgG in the sera of all 30 recipients exceeded the concentrations that were associated with protection. Furthermore, the median concentration of serum anti-toxin A IgG in the test group was 50-fold higher than the ELISA threshold associated with protection in the course of CDI. These findings suggest that the Sponsor's *C. difficile* Toxoid Vaccine is able to elicit a serum IgG response higher than that associated with natural infection. Additionally, ELISA and toxin neutralization assay (TNA) IgG levels to both toxins tended to be higher in the higher doses and adjuvanted formulations. All recipients in the 100 µg + aluminum containing adjuvant group mounted at least a 13-fold increase in anti-toxin A IgG within 14 days of first vaccine.

When used to treat patients with recurrent CDI (in H-030-006 study – 50 µg with schedule on Days 0, 7, 28, and 56), a similar formulation of the Sponsor's vaccine was also successful in preventing CDI recurrence for up to 6-month follow-up in 3 subjects who had chronic CDI recurrences and on prolonged antibiotic treatment (25). Two out of the 3 subjects showed an increase in anti-toxin A and B IgG. Both also developed cytotoxin neutralization activity against toxin A and B. The ability of serum to neutralize the cytotoxic activity of *C. difficile* toxins A and B generally coincided with increases in anti-toxin IgG levels as measured by ELISA.

Studies of the Sponsor's later formulations of the *C. difficile* vaccine have shown the vaccine to be highly immunogenic in targeted populations as measured by standard ELISA including fold-rise from baseline, as well as, toxin neutralizing capability.

Passive intravenous immunotherapy with human monoclonal antibodies against toxins A and B has also been shown to be well tolerated and effective in protecting patients from recurrence of CDI (26) (27) (28). When combination monoclonal antibodies to toxin A (CDA1) and B (CDB1) were administered to symptomatic CDI patients in conjunction with standard antimicrobial therapy, recurrence rates were reduced by 72% (recurrence: 7/101=7% Mab, 25/99=25% placebo) (28).

Taken together, the effectiveness of serum anti-toxin antibodies in protection from symptoms in colonized individuals and serum anti-toxin A and B antibodies in reduction of recurrent disease support the hypothesis that systemic humoral immunity against *C. difficile* toxins will prevent the manifestations of CDI in humans. It is anticipated that through active vaccination, the *C. difficile* Toxoid Vaccine will elicit broader anti-toxin responses and immunological memory.

1.2 Background of the Investigational Product

Background Studies

Over 8 years of clinical evaluation using a bivalent toxoid *C. difficile* vaccine, there have been no safety concerns identified in almost 900 subjects who received the ACAM-CDIFF vaccine formulation (N=688; at 50 µg or 100 µg with or without adjuvant) or closely related formulations.

Early Phase I and Phase II Studies

Five Phase I studies (H-030-005, H-030-006, H-030-008 (29) (30), H-030-009 (29) (30), and H-030-010) and 1 Phase II study (H-030-007) were conducted with early formulations of *C. difficile* toxoid vaccine (Original Vaccine and Modified Vaccine). During this development period, active vaccine (either adjuvanted with 400 µg or 750 µg aluminum or unadjuvanted) was administered to a total of 189 healthy adult and elderly subjects (18 to 55 years, and ≥ 65 years). An additional 47 subjects received placebo.

In particular, in these 6 clinical studies, there were no deaths and no related serious adverse events (SAEs). Two subjects reported 3 unrelated SAEs: 1 subject experienced 2 SAEs, pneumonia and cardiomyopathy; and the other subject experienced hemorrhagic diverticulitis. Including the subject who reported the 2 unrelated SAEs, a total of 3 subjects (1.5%) discontinued from these clinical studies, 1 subject withdrew due to a related severe adverse event (AE) (injection site reaction), and 1 subject discontinued due to an unrelated AE (increased platelet count, not considered severe). A total of 9 (4.6%) severe AEs were reported by subjects in the early studies. These included the following 6 unrelated severe AEs: abdominal pain (2); pelvic pain (1); headache (1); raised white cells (1); and proteinuria (1). Three severe local reactions were considered to be related and included: injection site erythema (≥ 5.0 cm) and injection site swelling after the second dose at 100 µg; injection site erythema (≥ 5.0 cm), and injection site induration after the third dose at 10 µg; and injection site erythema (≥ 5.0 cm), injection site induration, and injection site edema after the third dose at 2 µg. In 2/3 subjects who experienced severe injection site reactions, the events resolved without treatment and the subjects continued in the study.

Overall, in these early studies, the *C. difficile* Toxoid Vaccine was well tolerated and raised no safety concerns. The vaccine was also strongly immunogenic and supported the decision to progress to the more recent Phase II studies.

Recent Phase II Studies using ACAM-CDIFF Vaccine

Two Phase II studies, H-030-011 and H-030-012, were conducted with the ACAM-CDIFF vaccine formulation. In the H-030-011 study, of a total of 116 subjects aged ≥ 18 years were enrolled. Three subjects did not receive an injection; therefore, 75 subjects received ACAM-CDIFF vaccine and 38 subjects received placebo. In the H-030-012 study, a total of 455 subjects

aged 40 to 75 years were randomized, 405 received ACAM-DIFF Vaccine, and 50 received placebo in Stage I; 205 subjects received vaccine in Stage II. All SAEs were considered to be unrelated to the ACAM-CDIFF vaccine.

The results from these Phase II studies corroborated the findings from our early clinical studies: namely, an immune response in all vaccine groups compared to placebo; and a safety profile for elderly and adult subjects with recurrent, or at risk for, CDI that is comparable to the safety profile described for healthy volunteers.

H-030-011 Study (Safety and Immunogenicity in Symptomatic CDI Patients)

The H-030-011 study was a Phase II, randomized, double-blind, controlled, multi-center trial conducted in the UK and US. Adults aged ≥ 18 years who had confirmed primary CDI were enrolled within 12 days of laboratory diagnosis (using toxin antibody ELISA/EIA or PCR).

The primary objective of this study was to evaluate the potential for the vaccine to prevent recurrence of CDI when administered to patients currently experiencing symptomatic CDI. Recurrence was assessed in the 9 weeks after the third dose of vaccine in subjects who had experienced a primary CDI within 12 days before the first injection. Recruitment to this study was difficult and enrollment was stopped after randomization of 116 out of 612 planned subjects; therefore, all results were descriptive and no efficacy outcome was obtained.

Subjects were randomized to 1 of 4 groups: Group 1, placebo (39 subjects); Group 2, 50 μg plus adjuvant (17 subjects); Group 3, 100 μg plus adjuvant (42 subjects); and Group 4, 100 μg without adjuvant (18 subjects).

Immunogenicity

The conclusions drawn from the data must be viewed in the context of the relatively small sample size. A treatment effect was noted among all vaccine groups compared to placebo. There was a consistent rise in geometric mean concentrations (GMCs)/geometric mean titers (GMTs) in the vaccine groups for both toxins at each sampling day with the highest GMCs/GMTs seen at Day 42. For toxins A and B at Day 42, subjects administered the high dose (100 μg with or without adjuvant) had the highest GMC responses, while GMT responses to both toxins were more variable across groups and doses. In subjects aged ≥ 65 years, the highest GMC/GMT responses for both toxins were observed in the 100 μg group.

Safety

Sanofi Pasteur and the Independent Data Monitoring Committee (IDMC) review of this study concluded that the vaccine was safe and well tolerated. There were no related SAEs or deaths reported for subjects in any treatment group. Seven subjects died during the trial and Investigators considered all 7 deaths unrelated to vaccination. Although a higher percentage of subjects in the vaccine treatment groups reported solicited injection site and systemic reactions than in the placebo group, the reactogenicity profile was similar to that of other alum-adjuvanted vaccines. In general, the safety overviews were similar for subjects aged 18 to 64 years and subjects aged ≥ 65 years.

A total of 5.3% (4/75) of subjects discontinued from the pooled treatment due to AEs after any vaccine injection, and 2.6% (1/38) of subjects who had received placebo discontinued the study.

SAEs after any vaccine injection up to an including Day 91 were reported by 24.0% (18/75) of subjects in the pooled vaccine group and 26.3% (10/38) of subjects who had received placebo. SAEs after any vaccine injection at any time during the study were reported by 32.0% (24/75) of subjects in the pooled vaccine group and 34.2% (13/38) of subjects administered placebo. No SAEs were considered related to vaccination.

Overall, solicited injection site reactions were reported by 84.5% (60/71) of subjects in the pooled vaccine treatment group compared to 27.0% (10/37) of subjects in the placebo treatment group. The solicited injection site reaction reported most commonly was injection site tenderness. Solicited systemic reactions (without bowel movement and loose/watery bowel) after any vaccine injection were reported by 81.7% (58/71) of subjects in the pooled vaccine treatment group compared to 59.5% (22/37) of subjects in the placebo group. The solicited systemic reaction reported most commonly (without bowel movement and loose/watery bowel) was abdominal pain.

H-030-012 (Safety and Immunogenicity in Subjects at Risk for CDI)

The Phase II trial H-030-012 was a randomized, placebo-controlled study conducted in the US in adult subjects aged 40 to 75 years who were at risk for CDI due to either impending hospitalization within 60 days of enrollment or current or impending residence in a long-term care facility or rehabilitation facility within 60 days of enrollment.

During Stage I of the study, ACAM-CDIFF vaccine (N=405) or placebo (N=50) were administered on Days 0, 7 and 30 to the study groups as follows: Group 1, 50 μg antigen with adjuvant; Group 2, 50 μg antigen without adjuvant; Group 3, 100 μg antigen with adjuvant; Group 4, 100 μg antigen without adjuvant; Group 5, placebo (normal saline).

Analysis of the Day 60 safety and immunogenicity information from Stage I of study H-030-012 was used by the Sponsor to determine the formulation, 100 μg + aluminum hydroxide adjuvant, which was studied in Stage II.

ACAM-CDIFF vaccine was administered to 205 subjects during Stage II in 2 dosing schedules (Days 0, 7, and 180 [Group 6] and Days 0, 30, and 180 [Group 7]). These data provide an opportunity to evaluate immune persistence, alternate dose schedules (2-dose schedule with a booster at Day 180), as well as biological safety laboratory tests. In addition, all eligible subjects who received 100 μg + aluminum hydroxide adjuvant in Stage I (Group 3 subjects) and all subjects in Stage II were asked to participate in an extension of the study for possibly up to 3 years after the last vaccination to further describe immunogenicity of the vaccine, confirmed CDI episodes, and occurrence of SAEs that could be considered related to vaccination and/or study procedure (i.e., the blood draw).

Stage I: Safety Assessment

Note: Safety data are presented up until Study Day 60.

There were no SAEs or deaths considered related to vaccination by the Investigator for subjects in any treatment group. Four subjects died due to SAEs. The most frequent reason for discontinuation before Day 60 was voluntary withdrawal not due to an AE. Two subjects (2.0% [2/102]) in Group 4 (high dose, 100 μg without adjuvant) discontinued the study due to an unrelated SAE; and 5 subjects (5.0% [5/101]) in Group 1 (low dose, 50 μg with adjuvant), 1 subject (1.0% [1/101]) in Group 2 (low dose, 50 μg without adjuvant), and 2 subjects (2.0%

[2/102]) in Group 4 (high dose, 100 μg without adjuvant) discontinued the study due to non-serious AEs.

- The number of solicited or unsolicited Grade 3 reactions was similar and minimal among all treatment groups.
- The solicited adverse reactions (ARs) and unsolicited AEs were generally Grade 1 in intensity, of short duration, did not lead to study discontinuations and were not considered clinically significant.
- There were more solicited injection site and systemic reactions in the vaccine treatment groups; however, the tolerability profile was acceptable and similar or better than the tolerability profiles of other licensed vaccines.
- Unsolicited, non-serious ARs in the System Organ Class of general disorders and administration site conditions, specifically for unsolicited injection site reactions, were reported by more subjects in the adjuvanted groups than the unadjuvanted groups; however, the tolerability profile was acceptable.
- Overall, subjects in the older age group (ages 65 to 75 years) did not experience increased solicited ARs or unsolicited AEs, and the safety summary of the older group was similar to that of younger subjects (ages 40 to 64 years). As with the younger subjects, there was a slightly higher number of older group subjects reporting AEs and ARs in the adjuvanted groups; however, the tolerability profile was acceptable in this group as well.
- The IDMC reviewed all the safety data in October 2011 and did not identify any safety concerns.

Stage I: Immunogenicity Assessment

- A treatment effect was noted among all vaccine groups compared to placebo.
- Immune responses in all treatment groups were robust and continued to increase at Day 60.
- There were more subjects who were seropositive at baseline for toxin B than for toxin A.
- The high dose induced the highest GMC/GMT value for the population aged 65 to 75 years, as measured by ELISA and TNA. Based on bootstrap analysis and as measured by ELISA, the 100 μg + aluminum hydroxide adjuvant produced the highest ranking. This was noted especially in the group aged 65 to 75 years.

The 100 μg + aluminum hydroxide adjuvant (Group 3) dose was selected by Sanofi Pasteur for progression to Stage II, given the tolerability profile was acceptable and the overall immune responses were considered preferable, particularly in the 65 to 75 year age group. This age group represents patients who are most burdened by CDI and will make up a large portion of those enrolled for the Phase III efficacy, immunogenicity, and safety study.

Stage II: Safety Assessment

Note: Safety data are presented through Study Day 210.

A total of 6 subjects discontinued due to SAEs (1 within 30 days after vaccine injection) and 2 subjects due to other AEs (within 30 days of vaccine injection). Across the groups, the most

common reason for discontinuation during Stage II was voluntary withdrawal not due to an AE, reported by 6.9% (7/101) of subjects in the 100 µg + aluminum hydroxide adjuvant treatment group, 6.8% (7/103) of subjects in the group on schedule Days 0, 7, 180, and 12.6% (13/103) of subjects in the group on schedule Days 0, 30, 180. In Groups 6 (schedule Days 0, 7, 180) and 7 (schedule Days 0, 30, 180) for which Day 180 was the last day of vaccination, there were 1.9% (2/103) and 3.9% (4/103), respectively, who were lost to follow-up, compared to 1.0% (1/101) of subjects in Group 3 for which Day 30 was the last day of vaccination.

Overall, the vaccine formulation (100 µg + aluminum hydroxide adjuvant) administered at 3 different schedules had an acceptable safety profile with no safety signals identified. The safety overview was similar between subjects aged 18 – 64 years and subjects ≥ 65 years old.

- The reactogenicity was somewhat increased in Groups 6 and 7 following the Day 180 or Day 210 vaccine administration; however, the tolerability profile was acceptable. The solicited injection site reaction reported most frequently was injection site pain. The solicited systemic reaction reported most frequently was myalgia.
- Solicited reactions (injection site and systemic) after each vaccine injection began within 3 days after vaccine injection, were of Grade 1 intensity, occurred for 1 to 3 days, and did not require any action.
- Unsolicited AEs were reported most frequently in the SOC of gastrointestinal disorders by subjects in each group. Few AEs were considered related to vaccination.
- There were few AEs (1 in each group) that led to study discontinuation within 30 days of vaccination.
- SAEs were evenly distributed across the vaccine groups and were based on the subjects pre-existing or ongoing medical conditions or worsening during planned hospitalization. No SAEs were considered related to vaccination by the Investigators.
- A total of 4 subjects died during the study. No death was considered related to vaccination by the Investigator. All of these subjects had multiple ongoing or aggravated medical conditions and were on various concomitant medications.
- Biological laboratory parameters were not unexpected for this population. Clinically significant laboratory findings identified during the study were associated with underlying medical conditions and were not considered related to vaccination by the Investigators.

Stage II: Immunogenicity Assessment

- The vaccine formulation of 100 µg + aluminum hydroxide adjuvant was immunogenic for the 3 vaccine injection schedules (Days 0, 7, 30; Days 0, 7, 180; Days 0, 30, 180) tested, at all timepoints, and for both adult (aged 40 to 64 years) and elderly (aged 65 to 75 years) subjects.
- Immune responses at Days 7 and 14 were similar for the 3 testing schedules in adult and elderly subjects.
- The ELISA GMCs and TNA GMTs were higher at Days 60 and 180 for subjects in Group 3 than in Group 6 or 7. As expected , subjects in Groups 6 and 7 had the highest GMCs and GMTs at Day 210, because they had been vaccinated 30 days prior on Day 180 (unlike subjects in Group 3).

- Seroconversion (measured either by ELISA or TNA) was higher on Days 60 and 180 for subjects in Group 3 than in Group 6 or 7.
- Based on the bootstrap ranking analysis which focused principally on the immune response over the first 60 day period, Group 3 was chosen as the best schedule. Importantly, when viewed over the 180-day period during which maximum vaccine protection would be desired in patients who have recently entered a defined risk period for CDI, administration of 3 doses with a 0, 7 and 30 day regimen (Group 3) overall provided the best immune response as compared with the other 2 regimens (Groups 6 or 7) taking into consideration the immune responses measured over Days 30, 60 and 180. This period represents a period when patients are likely to be of greatest risk of developing CDI.
- [redacted]

1.3 Potential Benefits and Risks

1.3.1 Potential Benefits to Subjects

There is a possibility that the vaccine may prevent an episode of CDI that otherwise might have occurred.

1.3.2 Potential Risks to Subjects

As with any vaccination, subjects may experience pain, swelling, redness, or itching at the injection site. Rarely, a subject may experience an allergic reaction, which produces rash, hives, or difficulty breathing. Serious allergic reactions that can be life-threatening may occur.

In the recent 2 Phase II studies, the number of solicited or unsolicited Grade 3 reactions was similar and minimal among all treatment groups. The solicited ARs and unsolicited AEs were generally Grade 1 in intensity, of short duration, did not lead to study discontinuations and were not considered clinically significant. Solicited injection site pain and solicited systemic myalgia were reported most frequently in all vaccine treatment groups and the placebo group. The number and percentage of subjects who reported other solicited systemic reactions was balanced across vaccine treatment groups and after each injection. More information can be found in the Investigator's Brochure. See also Section 1.1.

Based on the human experience to-date that has occurred with the various formulations of *C. difficile* Toxoid Vaccine, no risks/potential risks have been identified from the safety data received so far. The safety and tolerability profile to date has been acceptable and similar or better than the tolerability profile of other licensed vaccines.

1.4 Rationale

This study will evaluate the efficacy of the candidate *C. difficile* vaccine to prevent primary symptomatic CDI in subjects at risk for CDI where there is a substantial unmet medical need.

Vaccine Dose and Schedule

The 100 µg + 400 µg aluminum/dose formulation, evaluated in the Phase II study H-030-012, was selected by the Sponsor with concurrence from the IDMC for progression to Phase III as the tolerability profile was acceptable and the overall immune responses were considered preferable, particularly in the group aged 65 to 75 years. As older individuals are likely to be a major portion of the target population for the administration of the *C. difficile* vaccine, choosing this dose is likely to provide the maximum vaccine protection in the Phase III study. There is also historical data noting that licensed toxoid adsorbed vaccines adjuvanted with aluminum salts induce adequate immune responses after fewer doses of toxoid. As with tetanus toxoid, the aluminum containing vaccine may be better at inducing responses in those without prior exposure and subsequent antibody production to toxin A or B (31). The selected schedule of Day 0, 7 and 30 demonstrated an immune response with an early boost at Day 7 and with the goal of obtaining protective immunity by Day 14. This immune profile best supports the use of a prophylactic *C. difficile* vaccine in circumstances where planned hospitalization for surgery is part of a patient's health management. In addition, it is more clinically feasible for patients to receive all three vaccines within a 30-day period than expect patients to return for their third and final vaccine at a later than 30 day timeframe.

Target Population

The selection of the population for the Phase III clinical study was based on the vaccine potency (likelihood of immune response) and logistical aspects (ability to vaccinate 15 days before the greatest exposure to *C. difficile*, uncomplicated identification of subjects by practitioners, and acceptability of the vaccine dose/schedule by the subject). We selected an enriched population with an estimated CDI incidence of at least 1.5% yearly, which we considered as the best compromise between increased CDI risk for a reasonable Phase III sample size and sizeable population for recruitment feasibility. This enrichment was based on the likelihood of a forthcoming hospitalization and antibiotic use. To select this population, a database analysis exercise was conducted in 2012 on the most recent data available (2010-2011) (32).

[redacted] This analysis corroborated age as an independent risk factor of CDI; the rate of CDI at 1 year was greater than 1.2% after 60 years. The number of antibiotic courses used in the last 60 days showed a linear dose response relationship with the risk of CDI (OR = 2.1 per additional course). In fact, this study revealed that co-morbidities alone may not be the best proxy for high risk population since people with co-morbidities who developed CDI were the ones with significantly higher antibiotic use. Previous hospital stay was a good predictor with a chance of a CDI event of 1.5% over 1 year for people with at least 2 hospital stays in the previous year. By combining the previous findings, we define an enriched population with co-morbidities defined by "people 50+ years with repeated hospital stays in the previous year and history of antibiotic use".

A second analysis focused on inpatient data in order to identify surgeries at highest risk of CDI. This analysis was performed on Premier inpatient database which covers 20% of the discharge in the US. We identified several families of surgeries with a risk of CDI > 1.5% before discharge

e.g. kidney/urinary tract/bladder, musculoskeletal, respiratory, and circulatory system. Most of them were associated with a high antibiotic use (> 70%) and significant length of stay (> 5 days). These rates are probably underestimated since a significant share of the CDI acquired during the surgery stay will appear within 1 month from discharge. Indeed, approximately 80% of the patients had a CDI occurrence within 2 months of discharge with most of the remaining cases occurring before 6 months after discharge (32) (33). This analysis allowed us to define a second set of eligible population among selected planned surgeries in patients aged 50+ years.

[redacted] (33). Even though risk of CDI were generally lower (because of a lower representation of at-risk and Medicare population), trends and findings were the same.

Primary Endpoint

The diagnosis of CDI should be based on a combination of clinical and laboratory findings (34). In alignment with current guidelines and key subject matter experts, the primary endpoint will be based on meeting both a clinical and laboratory definition. The clinical criteria will require at least 3 or more loose stools within a 24 hour or less time period that lasts for greater than 24 hours or the finding of pseudomembranous colitis on colonoscopy. The laboratory criteria require a stool sample positive for *C. difficile* by PCR in a subject meeting the clinical criteria as noted above. PCR has been extensively used and repeatedly validated in the diagnosis of CDI in a variety of settings. Overall, when compared to other CDI diagnostics, PCR has the best sensitivity and specificity (35).

The combination of strict clinical case criteria combined with an accurate, reproducible and centralized laboratory PCR assay utilized in a patients population at high risk for *C. difficile* and unlikely to have diarrhea attributable to other sources will yield an accurate assessment of incident CDI cases and improve upon the specificity of a molecular based diagnostic testing for etiologic confirmation in this vaccine trial.

Group Sequential Design with Interim Analysis

In this group sequential design, data are monitored throughout collection, and the decision to stop the trial can be made before all of the data are accrued (36). The design specifically allows for multiple testing during trial monitoring while still preserving the overall operating characteristics of the primary test for efficacy. Details are provided in Section 12.

S***econdary and Exploratory Objectives, Endpoints and Approach to Analysis***

While the primary analysis will be based on a MITT approach and will include all subjects who have received at least 1 injection, secondary objectives will also assess the efficacy of the candidate vaccine based on the number of injections received, the durability of efficacy up to 3 years after the third injection and the efficacy to prevent severe CDI cases. Additionally, immunogenicity to both toxins will be evaluated in a subset of 1650 subjects (at multiple timepoints and up to 3 years from last injection), randomly selected in a blinded manner at study start, and in approximately 250 subjects with CDI (at timepoints Days 0 and 60). The safety profile will be evaluated in all subjects that have received at least 1 injection.

[redacted]

[redacted]

Follow-up duration

Based on simulations, an average follow-up period of up to 3.0 years will be required in order to bound the probability of a failed trial at 1% for true VE between 60% and 90%. Here, we define a failed trial as one which does not accrue the required number of endpoints to trigger the interim or final analyses. If we enroll 16,500 subjects, we need to follow them for at least 2 years to have a low probability of a failed trial (less than 1%) under our base case design assumptions (e.g., endpoint incidence rate, loss to follow up, *C. difficile* prevalence among clinical cases). A follow-up duration of 3.0 years was selected to provide some robustness of the design to variations in those design assumptions.

2 Trial Objectives

2.1 Primary Objective

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

The endpoints for the primary objective are presented in Section 9.1.1.

2.2 Secondary Objectives

Efficacy:

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

Immunogenicity:

- To describe the immunogenicity to toxin A and toxin B:
  - in the subset (1650 out of 16,500) of subjects and in subjects with CDI (250) at Day 0 and Day 60 (± 14 days)

  and

  - in the subset (1650 out of 16,500) of subjects at Day 14 (+3 days), Day 30 (–3 days to +7 days), and every 6 months up to 3.0 years (± 14 days) after the third injection

Safety:

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

The endpoints for the secondary objectives are presented in Section 9.2.

2.3 [redacted]

[redacted]

3 Investigators and Trial Organization

This trial will be conducted in approximately 350 centers in the US and Canada and other selected countries in the various regions (Europe, Asia Pacific, and Latin America). The Principal Investigators and any sub-investigators at the individual sites will be coordinated by a Coordinating Investigator. As required by local regulations, there may be 1 Coordinating Investigator per country or 1 Coordinating Investigator per region. Details of the trial centers, the Investigators at each center, and the Coordinating Investigator are provided in the "List of Investigators and Centers Involved in the Trial" document.

An IDMC will be utilized throughout this trial. The committee will periodically assess the progress of the clinical trial and recommend to Sanofi Pasteur whether to continue, modify, or stop it. The IDMC will be responsible for the evaluation of the safety of *C. difficile* Toxoid Vaccine; a request that the code be broken may be made by the IDMC if needed to facilitate their assessment of safety (Section 6.4) and for making recommendations to the Sponsor on a medical and ethical basis. Also the IDMC and the unblinded independent statistician will have access to the unblinded analyses at the 4 interim analyses. At each of the interim analyses, the IDMC will review the unblinded data and advise to either continue the trial as planned or to stop the trial (for efficacy or for futility) using the statistical testing as primary guidance for their recommendation.

Stool testing by PCR for the primary endpoint and monitoring will be performed by a contract research organization (CRO).

Immunogenicity testing will all be performed at the Sanofi Pasteur Global Clinical Immunogenicity (GCI) laboratories in Swiftwater, PA, USA or at a Sanofi Pasteur-approved laboratory. [redacted]

4 Independent Ethics Committee / Institutional Review Board

Before the investigational product can be shipped to the investigational site and before the inclusion of the first subject, this protocol, the informed consent form (ICF), subject recruitment procedures, and any other written information to be provided to subjects must be approved by, and/or receive favorable opinion from, the appropriate Independent Ethics Committee (IEC) or Institutional Review Board (IRB).

The following procedures are described in general and may be adapted according to local regulations. In accordance with Good Clinical Practice (GCP), the Sponsor and/or Investigator are responsible for obtaining this approval and/or favorable opinion before the start of the trial. If the protocol is subsequently amended, approval must be re-obtained for each amendment. Copies of these approvals, along with information on the type, version number, and date of document, and the date of approval, must be forwarded by the Investigator to the Sponsor together with the composition of the IEC/IRB.

The Investigator or the Sponsor will submit written summaries of the status of the trial to the IEC/IRB annually, or more frequently if requested. According to the local IEC/IRB policy, either all SAEs or all SAEs occurring during the trial that are related to vaccination will be reported by the Investigator to the IEC/IRB.

5 Investigational Plan

5.1 Description of the Overall Trial Design and Plan

5.1.1 Trial Design

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

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

Risk Stratum 1:

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

  and

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

or

Risk Stratum 2:

- Is anticipated to have an in-patient hospitalization for a planned surgical procedure within 60 days of enrollment and the impending hospital stay is planned to be ≥ 72 hours (see Section 5.2.4, Inclusion Criteria).

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

The study is designed as an event-driven, group sequential protocol with 4 interim analyses at 50, 100, 135, 167 clinical endpoints (i.e., PCR-confirmed CDI episodes) and a final analysis when 250 clinical endpoints are reached.

The planned sample size is 16,500 subjects. Subjects will be randomly assigned in a 2:1 ratio to receive either vaccine or placebo. Vaccine or placebo will be administered in a 3-dose schedule on Days 0, 7, and 30. At the time of group assignment, 1650 subjects (10% of total enrollment) will be randomly assigned to an immunogenicity subset; and 3300 subjects (20% of total enrollment) will be randomly assigned to a reactogenicity subset (see also Section 6.5).

All subjects will provide blood samples for immunogenicity assessment at Day 0 (pre-injection) and Day 60. Subjects in the immunogenicity subset from both study groups will also provide blood samples at Day 14, pre-injection at Day 30, and every 6 months through the planned follow-up periods (i.e., up to 3.0 years after the third injection).

In the reactogenicity subset, solicited ARs will be collected for 6 days following each injection. Unsolicited AEs will be collected for all subjects from Day 0 to Day 60. All SAEs will be collected through the end of the follow-up period.

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

Analyses of trial futility (non-efficacy) will be performed at the first 2 interim analyses (See Table 5.1). The study may be stopped if either of those analyses provides robust and compelling evidence that meaningful levels of vaccine efficacy (VE) will not be demonstrated.

[redacted]

Analyses of efficacy will be performed at the second, third, and fourth interim analyses as well as the final analysis, with the total one-sided type 1 error rate at 0.025. The nominal significance levels for the 3 efficacy interim analyses are 0.01, 0.0035 and 0.0032, respectively, as determined by the corresponding proportion of information assessed at each interim analysis (number of new cases at each interim / 250 * 0.025). The remaining alpha (0.0083) after the interim analyses will be used at the final analysis. Based on trial simulations, if the true VE is 60%, there is an estimated 45% probability to reject the primary null hypothesis at the second interim (first interim for efficacy).

Table 5.1: Interim analyses and associated cumulative alpha spending

Interim Analysis	Futility	Efficacy: cumulative alpha (type 1 error rate) spent
Approximately 50 cases	Yes	
Approximately 100 cases	Yes	0.01
Approximately 135 cases		0.0135
Approximately 167 cases		0.0167
Final Analysis		0.025

[redacted]

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

5.1.2 Justification of the Trial Design

The trial is designed as an observer-blind study. According to local requirements, the vaccine preparer and administrator may be unblinded to treatment assignment due to the steps necessary

for vaccine preparation. The vaccine requires addition of diluent to the lyophilized powder before extraction of the vial contents to the syringe. The vaccine solution is visually distinct from the placebo solution; therefore, the individual(s) responsible for the vaccine preparation and/or administration will be unblinded and all personnel involved in safety assessments will be blinded to group assignment.

The vaccine being administered in H-030-014 is a very similar formulation that was used in Stage II of the Phase II study H-030-012 and in the schedule of Days 0, 7, and 30. As is typical in a development program, product enhancement and improvements occur as experience and data are gained from pre-clinical and clinical data. [redacted]

Placebo, rather than a benefit licensed vaccine, will be used for comparison to vaccine to allow safety comparisons between vaccine and placebo as there are no other licensed CDI vaccines on the market.

The current vaccine formulation has been tested in 2 recent Phase II clinical trials, and has been shown to be both immunogenic and safe. The route of administration (IM) has been used in all clinical trials to date.

An IDMC will be used in this study. The IDMC will be involved in reviewing the SAE cases due to deaths or life-threatening SAEs that are considered related to vaccine. Additionally, the trial will be monitored by the IDMC, as they will be the only group with access to the unblinded analyses at the 4 interim analyses. At each of the interim analyses, the IDMC will review the unblinded data and advise to either continue the trial as planned or to stop the trial (for efficacy or for futility) using statistical testing as primary guidance for their recommendation.

Collection of SAE cases will continue throughout the trial. For analysis purposes, the primary safety evaluation period will include SAEs reported up to 6 months after the last dose of study vaccine. SAEs with an outcome of death or non-fatal SAEs leading to hospitalization will also be submitted for review and adjudication by a blinded external Adjudication Committee, as per IDMC request. The procedures governing the function of this Committee are outlined in a separate Manual of Operations included with the IDMC Charter.

For additional trial design information, please see Section 1.4.

5.1.3 Trial Plan

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

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

For subjects in the main study, blood (20 mL/bleed) will be collected before injection at Visit 1 (Day 0) and at Visit 4 (60 days [+14] days after injection).

At enrollment, 10% of subjects (1650 out of 16,500) will be randomly assigned by IVRS/IWRS to an immunogenicity subset. For these subjects, blood (20 mL/bleed) for serologic testing will be

collected before injection at Visit 1 (Day 0) and at Visit 3 (Day 14), Visit 4 (Day 30), Visit 5 (Day 60), Visit 6 (Day 210), and then every 6 months as long as the subject is in the trial.

Also at enrollment, 20% of subjects (3300 out of 16,500) will be randomly assigned by IVRS/IWRS to a reactogenicity subset. These subjects will be asked to record solicited reactions for 6 days after each injection.

All subjects will record unsolicited AEs that occur from Day 0 through Day 60 (30 days after the last injection). All subjects will be followed for safety for at least 6 months after the last injection.

Subjects will be contacted on Day 210 (±14 days) and up to 3.0 years (±14 days) to ascertain whether any additional SAEs not already reported occurred since the last contact. All SAEs (related and unrelated) will be collected in all subjects from Day 0 through the end of the follow-up period. During the follow-up period, subjects will be instructed to contact the clinical site if they are hospitalized or admitted to a long-term care or rehabilitation facility, or if they experience an AE that might be considered serious (Section 10).

All subjects will be followed for the follow-up period (up to 3.0 years after the third injection) for occurrence of repeated episodes of loose stools and provision of a stool sample. For all subjects, there will be a weekly contact (telephone, home or clinic visit) for 4 weeks after enrollment (Day 0 through Day 30) or following discharge from a hospital or long-term care or rehabilitation facility, to remind subjects to report repeated episodes of loose stools and provide stool samples, if applicable. Following the first 4 weeks, subjects will be contacted every 2 weeks for the duration of the study and follow-up period. The method of contact may be by telephone, text message, email, and/or home visit; however, there must be subject contact via telephone or home visit once a month.

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

Additionally, a quality of life questionnaire will be completed following a repeated episode of loose stools for which a sample is collected. When possible, subjects will complete the survey on their own or via telephone interview with the site staff. Two other quality of life questionnaires will be completed within 5 to 6 and 8 to 9 weeks after the beginning of each episode. For subjects in the immunogenicity subset, a quality of life questionnaire will also be completed at Visits 3, 6, 8, and 10. The questionnaire may be completed by the subject or may be administered and recorded by site staff via telephone or may be completed by a proxy in case the subject's condition is such that he or she is not able to answer.

5.1.4 Visit Procedures

5.1.4.1 Main Population (N=14,850 out of a total of 16,500)

Note: see Section 5.1.4.2 for the visit procedures for subjects in the immunogenicity subset (N=1650)

5.1.4.1.1 Visit 1 (Day 0): Inclusion, Randomization, Collection of Blood Sample, and Vaccination

Note: Pregnancy testing and confirmation that the subject meets inclusion/exclusion criteria, must be completed on the day of vaccination (Day 0). If an ICF has been signed and dated, a physical examination and other study activities may be performed up to 7 days prior to Day 0.

1) Provide the subject information about the trial (including specifics concerning stool sample collection and duration of contacts), obtain written informed consent (if not already done), and give him/her a signed copy of the consent form.
2) Complete review of inclusion and exclusion criteria for eligibility.
3) Collect and record demographic data in the source document.
4) Obtain a urine sample for pregnancy testing (women of childbearing potential only). If the test is positive, enrollment cannot proceed.
5) Interview the subject on any past or current significant personal medical history and, for subjects aged > 65 years, Frailty-associated conditions. Record the information in the source document.
6) Perform a body system targeted physical examination prior to vaccination based on the subject's medical history and the examiner's medical judgment. The purpose is to ensure proper assessment of the subject, his/her baseline health status, and study qualification. Record the findings in the source documentation.
7) Take subject's temperature and record in the source document.
8) Record all concomitant medications in the source document. Specifically ask about and record any antibiotics taken within 30 days of enrollment, including start and stop dates.
9) Call the IVRS/ interactive web response system (IWRS) for randomization to a study group, potential assignment to immunogenicity subset or reactogenicity subset, assignment of a subject number, and designation of a unique product kit number.
10) If the subject is eligible for inclusion in the study, obtain the first blood sample (20 mL) and document this in the source document. See Section 7.1 for detailed instructions.
11) Prepare and administer the assigned study vaccine or placebo (Dose 1). Document this in the source documents and the eCRF. Include the date, site of injection (deltoid muscle), side (left or right), and route of injection (IM).

12) Staff blinded to group assignment will keep the subject under observation for 30 minutes, for safety surveillance. If any adverse reactions (ARs) occur, record them in the source document and the eCRF. If an SAE occurs, it must be reported using the eSAE reporting form.

13) Provide the first diary card (DC1) to all subjects for recording unsolicited AEs and concomitant medications. For all subjects, provide a digital thermometer. The thermometer is used to measure body temperature. (All subjects will record their temperature at the time of episodes of loose stools. Subjects in the reactogenicity subset will also use the thermometer to record the solicited systemic reaction of fever.) For subjects in the reactogenicity subset, also provide a ruler and instructions for their use. For these subjects, the diary card will have pages to record any solicited reactions (both injection site and systemic) over the next 6 days. The ruler is used to measure the size of any injection site reactions. Instruct all subjects to bring DC1 to the site at Visit 2.

14) Provide the memory aid for recording loose stools (MA-LS). Explain that the MA-LS will be used to record episodes of loose stools including date and time of each episode until cessation of event, time and date of episode, and indicate if stool collected. Remind the subject to take his/her temperature at the time he/she experiences loose stools and to record the temperature on the MA-LS.

15) Provide the subject with a container for the collection of stool, and instruct him/her how to collect a sample in the event of repeated episodes of loose stools. Provide the subject with a storage container to be used to maintain the stool sample until delivery or pick-up. Remind the subject to contact the clinical site if he experiences repeated episodes of loose stools and a stool sample is collected.

16) Provide the subject with written instructions explaining contacts during the surveillance period at home and/or during a hospitalization or admission to a long-term care or rehabilitation facility, and concerning stool sample collection.

17) If the subject experiences loose stools and a stool sample is provided, the study staff will ask the subject 3 times to complete the EQ-5D-3L quality of life questionnaire. This will occur at the time of the episode of loose stools and 5 to 6 and 8 to 9 weeks after the beginning of the episode. If the subject's condition is such that he or she cannot answer the questionnaire, a proxy can answer in his or her place. If the subject, or proxy, is unable to complete the questionnaire, the study nurse may ask the subject, or proxy, the questions over the phone and appropriately document the responses.

18) Schedule Visit 2 (7 [+3] days).

19) Remind the subject to notify the site if they are hospitalized or experience an AE that might be considered serious.

20) Complete the relevant eCRF pages for this visit.

5.1.4.1.2 Visit 2 (7 [+3] days after Visit 1): Collection of Safety Information and Vaccination

1) Check whether the subject experienced any SAE since the previous visit. If an SAE occurred, follow the instructions in Section 10 for reporting it.

2) Review the information in DC1 with the subject, including unsolicited AEs and concomitant medications or therapy administered since the first vaccination at Visit 1. For subjects in the reactogenicity subset, also review the solicited reactions. Collect DC1. Record any new findings, information on any admission to a healthcare facility, any additional information, and/or discrepancies between the diary card and the interview content in the source documents.
3) Perform a body system targeted physical examination prior to vaccination based on the subject's medical history and the examiner's medical judgment.
4) Check for any temporary or definitive contraindications to vaccination.
5) Take subject's temperature and record in the source document.
6) Obtain a urine sample for pregnancy testing (women of childbearing potential only). If the test is positive, vaccination cannot proceed.
7) Call the IVRS/ IWRS for unique product kit number.
8) Prepare and administer the assigned study vaccine or placebo (Dose 2) into the arm opposite to that used for the first, if possible. Document this in the source documents. Include the date, site of injection (deltoid muscle), side (left or right), and route of injection (IM).
9) Staff blinded to group assignment will keep the subject under observation for 30 minutes for safety surveillance. If any ARs occur, record them in the source document. If an SAE occurs, it must be reported using the eSAE reporting form.
10) Provide the second diary card (DC2) and instructions for its use. This diary card contains pages to report unsolicited AEs and concomitant medications for the day of vaccination through 30 days after vaccination. For subjects in the reactogenicity subset, there will be pages to report solicited reactions for the day of vaccination though 6 days after vaccination. Instruct the subject to bring DC2 to the site at Visit 3.
11) Remind the subject to record loose stools on the MA-LS. Explain again that the MA-LS will be used to record episodes of loose stools including date and time of each episode until cessation of event, time and date of episode, and indicate if stool collected. Remind the subject to take his/her temperature at the time he/she experiences loose stools and to record the temperature on the MA-LS.
12) Provide the subject with a container for the collection of stool if a new container is needed and instruct him/her how to collect a sample in the event of repeated episodes of loose stools. Remind the subject to contact the clinical site if he experiences repeated episodes of loose stools and a stool sample is collected. The site will review MA-LS information with the subject and record dates and times of loose stool episodes, and temperature at the time of the episodes in source documents.
13) If the subject experiences loose stools and a stool sample is provided, the study staff will ask the subject 3 times to complete the EQ-5D-3L quality of life questionnaire. This will occur at the time of the episode of loose stools and 5 to 6 and 8 to 9 weeks after the beginning of the episode. If the subject's condition is such that he or she cannot answer the questionnaire, a proxy can answer in his or her place. If the subject or proxy, is unable to complete the

questionnaire, the study nurse may ask the subject, or proxy, the questions over the phone and appropriately document the responses.

14) Schedule Visit 3 (30 [-3 to 7] days).

15) Remind the subject to notify the site if they are hospitalized or experience an AE that might be considered serious.

16) Complete the relevant eCRF pages for this visit.

5.1.4.1.3 Visit 3 (30 [-3 to 7] days after Visit 1): Collection of Safety Information and Vaccination

1) Check whether the subject experienced any SAE since the previous visit. If an SAE occurred, follow the instructions in Section 10 for reporting it.

2) Review the information in DC2 with the subject, including unsolicited AEs and concomitant medications or therapy administered since the second vaccination at Visit 2. For subjects in the reactogenicity subset, also review solicited reactions. Collect DC2. Record any new findings, information on any admission to a healthcare facility, any additional information, and/or discrepancies between the diary card and the interview content in the source documents.

3) Perform a body system targeted physical examination prior to vaccination based on the subject's medical history and the examiner's medical judgment.

4) Check for any temporary or definitive contraindications to vaccination.

5) Take subject's temperature and record in the source document.

6) Obtain a urine sample for pregnancy testing (women of childbearing potential only). If the test is positive, vaccination cannot proceed.

7) Call the IVRS/ IWRS for unique product kit number.

8) Prepare and administer the assigned study vaccine or placebo (Dose 3) into either arm. Document in the source documents the date, site of injection (deltoid muscle), side (left or right), and route of injection (IM).

9) Staff blinded to group assignment will keep the subject under observation for 30 minutes for safety surveillance. If any ARs occur, record them in the source document and the eCRF. If an SAE occurs, it must be reported using the eSAE reporting form.

10) Provide the third diary card (DC3) and instructions for its use. This diary card contains pages to report unsolicited AEs and concomitant medications for the day of vaccination through 30 days after vaccination. For subjects in the reactogenicity subset, there will be pages to report solicited reactions for the day of vaccination through 6 days after vaccination. Instruct the subject to bring DC3 to the site at Visit 4.

11) Remind the subject to record loose stools on the MA-LS. Explain again that the MA-LS will be used to record episodes of loose stools including date and time of each episode until cessation of event, time and date of episode, and indicate if stool collected. Remind the

subject to take his/her temperature at the time he/she experiences loose stools and to record the temperature on the MA-LS.

12) Provide the subject with a container for the collection of stool if a new container is needed, and instruct him/her how to collect a sample in the event of repeated episodes of loose stools. Remind the subject to contact the clinical site if he experiences repeated episodes of loose stools and a stool sample is collected. The site will review MA-LS information with the subject and record dates and times of loose stool episodes, and temperature at the time of the episodes in source documents.

13) If the subject experiences loose stools and a stool sample is provided, the study staff will ask the subject 3 times to complete the EQ-5D-3L quality of life questionnaire. This will occur at the time of the episode of loose stools and 5 to 6 and 8 to 9 weeks after the beginning of the episode. If the subject's condition is such that he or she cannot answer the questionnaire, a proxy can answer in his or her place. If the subject or proxy, is unable to complete the questionnaire, the study nurse may ask the subject, or proxy, the questions over the phone and appropriately document the responses.

14) Schedule Visit 4 (60 [+14] days).

15) Remind the subject to notify the site if they are hospitalized or experience an AE that might be considered serious.

16) Remind the subject that he/she will be contacted approximately every 2 weeks.

17) Complete the relevant eCRF pages for this visit.

5.1.4.1.4 Visit 4 (60 [+14] days after Visit 1): Collection of Safety Information and Collection of Blood Sample

1) Check whether the subject experienced any SAE since the previous visit. If an SAE occurred, follow the instructions in Section 10 for reporting it.

2) Review the information in DC3 with the subject, including unsolicited AEs and concomitant medications or therapy administered since the third vaccination at Visit 3. For subjects in the reactogenicity subset, also review the solicited reactions. Collect DC3. Record any new findings, information on any admission to a healthcare facility, any additional information, and/or discrepancies between the diary card and the interview content in the source documents.

3) Obtain a blood sample (20 mL). Document this in the source document. See Section 7.1 for detailed instructions.

4) Provide the memory aid (MA) and instructions for its use.

5) Provide the subject with a container for the collection of stool if a new container is needed, and instruct him/her how to collect a sample in the event of repeated episodes of loose stools. Remind the subject to contact the clinical site if he experiences repeated episodes of loose stools and a stool sample is collected. The site will review MA-LS information with the subject and record dates and times of loose stool episodes, and temperature at the time of the episodes in source documents.

6) If the subject experiences loose stools and a stool sample is provided, the study staff will ask the subject 3 times to complete the EQ-5D-3L quality of life questionnaire. This will occur at the time of the episode of loose stools and 5 to 6 and 8 to 9 weeks after the beginning of the episode. If the subject's condition is such that he or she cannot answer the questionnaire, a proxy can answer in his or her place. If the subject or proxy, is unable to complete the questionnaire, the study nurse may ask the subject, or proxy, the questions over the phone and appropriately document the responses.
7) Remind the subject that he/she will be contacted approximately every 2 weeks.
8) Remind the subject to notify the site if they are hospitalized or experience an AE that might be considered serious.
9) Complete the relevant eCRF pages for this visit.

5.1.4.1.5 Contact (~210 [±14] days after Visit 1): Safety Follow-up

1) Review MA and MA-LS, including all AEs and Category 2 and 3 concomitant medications with the subject.
2) Determine if an SAE occurred, and if so, follow the instructions in Section 10 for reporting it. All SAEs will be collected through the end of the follow-up period. The site will review MA-LS information with the subject and record dates and times of loose stool episodes in source documents.
3) Ask the subject whether he/she needs another container for the collection of stool, and instruct him/her how to collect a sample in the event of repeated episodes of loose stools. Remind the subject to contact the clinical site if he experiences repeated episodes of loose stools and a stool sample is collected. Remind the subject to take his/her temperature at the time he/she experiences loose stools and to record the temperature on the MA-LS.
4) If the subject experiences loose stools and a stool sample is provided, the study staff will ask the subject 3 times to complete the EQ-5D-3L quality of life questionnaire. This will occur at the time of the episode of loose stools and 5 to 6 and 8 to 9 weeks after the beginning of the episode. If the subject's condition is such that he or she cannot answer the questionnaire, a proxy can answer in his or her place. If the subject or proxy, is unable to complete the questionnaire, the study nurse may ask the subject, or proxy, the questions over the phone and appropriately document the responses.
5) Remind the subject that he/she will be contacted approximately every 2 weeks throughout the remainder of the study (see Section 5.1.4.4).
6) Remind the subject to notify the site if they are hospitalized or admission to a long-term care or rehabilitation facility, or experience an AE that might be considered serious.
7) Complete the relevant eCRF pages for this visit.

5.1.4.1.6 Contact (every 2 weeks up to 3.0 years after Visit 1): Safety and Efficacy Follow-ups

1) Review MA, including all AEs and Category 2 and 3 concomitant medications with the subject.
2) Determine if an SAE occurred, and if so, follow the instructions in Section 10 for reporting it. SAEs considered related or unrelated to vaccination and/or study procedures will be collected throughout the follow-up period.
3) Review MA-LS and any recorded repeated episodes of loose stools with the subject. The site will review MA-LS information with the subject and record dates and times of loose stool episodes, and temperature at the time of the episodes in source documents.
4) Ask the subject whether he needs another container for the collection of stool, and instruct him/her how to collect a sample in the event of repeated episodes of loose stools. Remind the subject to contact the clinical site if he experiences repeated episodes of loose stools and a stool sample is collected. Remind the subject to take his/her temperature at the time he/she experiences loose stools and to record the temperature on the MA-LS.
5) Remind the subject that he/she will be contacted approximately every 2 weeks throughout the remainder of the study
6) Remind the subject to notify the site of any hospitalization or admission to a long-term care or rehabilitation facility.
7) Remind the subject to notify the site if they are hospitalized or experience an AE that might be considered serious.
8) Complete the relevant eCRF pages for this visit.
9) At time of final contact, complete the trial termination record.

5.1.4.2 Immunogenicity Subset (N=1650 out of a total of 16,500)

5.1.4.2.1 Visit 1 (Day 0): Inclusion, Randomization, Collection of Blood Sample, and Vaccination

Follow the same steps listed in Section 5.1.4.1.1. During the IVRS/IWRS call in step 12, 10% of the subjects will be randomized to the immunogenicity subset and will be randomized to a vaccine group and assigned a subject number.

5.1.4.2.2 Visit 2 (7 [+3] days after Visit 1): Collection of Safety Information and Vaccination

Follow the same steps listed in Section 5.1.4.1.2.

5.1.4.2.3 Visit 3 (14 [+3] days) after Visit 1): Collection of Safety Information and Collection of Blood Sample

Follow the same steps listed in Section 5.1.4.1.4, with the following exceptions:

- The information in DC2 will be reviewed. DC2 will be returned to the subject.
- A MA will not be provided to the subject at this visit.
- Visit 4 (30 [-3 to 7] days) will be scheduled.

And with the following addition:

- Ask the subject to fill in EQ-5D-3L quality of life questionnaire while on site and return it to the staff while on site.

5.1.4.2.4 Visit 4 (30 days [-3 to 7] days after Visit 1): Collection of Safety Information, Collection of Blood Sample, and Vaccination

Follow the same steps listed in Section 5.1.4.1.2, with the following additions:

- DC2 will be reviewed and then collected.
- The third blood sample (20 mL) will be obtained and documented in the source document.
- The third injection (Dose 3) will be prepared and administered.
- DC3 will be distributed, to be brought back to the site at Visit 5.
- Visit 5 (60 [+14] days after Visit 1) will be scheduled.

5.1.4.2.5 Visit 5 (60 [+14] days after Visit 1): Collection of Safety Information and Collection of Blood Sample

Follow the same steps listed in Section 5.1.4.1.4, with the following modification:

- These steps will be followed after the third injection at Visit 4 (Section 5.1.4.2.4).

5.1.4.2.6 Visit 6 through Visit 11 (every 6 months up to 3.0 years): Collection of Safety Information, Collection of Blood Sample, and Trial Termination Record

1) At Visit 6, review MA, including all AEs and Category 2 and 3 concomitant medications with the subject and collect information on any admission to a healthcare facility.
2) Determine if an SAE occurred, and if so, follow the instructions in Section 10 for reporting it. All SAEs will be collected through the end of the follow-up period.
3) After Visit 6, review MA and all AEs with the subject and collect information on any admission to a healthcare facility. Determine if an SAE occurred, and if so, follow the instructions in Section 10 for reporting it. SAEs considered related or unrelated to vaccination and/or study procedures will be collected throughout the surveillance period.
4) Review MA-LS and any recorded repeated episodes of loose stools with the subject. The site will review MA-LS information with the subject and record dates and times of loose stool episodes, and temperature at the time of the episodes in source documents.
5) Obtain a blood sample (20 mL); these will be BL5 through BL10. Document this in the source document. See Section 7.1 for detailed instructions.

6) Ask the subject whether he needs another container for the collection of stool, and instruct him/her how to collect a sample in the event of diarrhea. Remind the subject to contact the clinical site if he experiences repeated episodes of loose stools and a stool sample is collected. Remind the subject to take his/her temperature at the time he/she experiences loose stools and to record the temperature on the MA-LS.
7) If the subject experiences loose stools and a stool sample is provided, the study staff will ask the subject 3 times to complete the EQ-5D-3L quality of life questionnaire. This will occur at the time of the episode of loose stools and 5 to 6 and 8 to 9 weeks after the beginning of the episode. If the subject's condition is such that he or she cannot answer the questionnaire, a proxy can answer in his or her place. If the subject, or their proxy, is unable to complete the questionnaire, the study nurse may ask the subject, or proxy, the questions over the phone and appropriately document the responses.
8) Remind the subject that he/she will be contacted approximately every 2 weeks between the clinic visits throughout the remainder of the study.
9) Remind the subject to notify the site of any hospitalization or admission to a long-term care or rehabilitation facility.
10) Remind the subject that the site personnel will make contact approximately 5 months after each visit to schedule an appointment for the next visit to obtain a blood sample.
11) Complete the trial termination record at Visit 11.
12) At Visits 3, 6, 8, and 10, ask the subjects in the immunogenicity subset to fill in EQ-5D-3L quality of life questionnaire while on site and return it to the staff while on site.
13) Complete the relevant eCRF pages for the visit.

5.1.4.3 Reactogenicity Subset (N=3300 out of a total of 16,500)

During the IVRS/IWRS call at Visit 1, the subject may be randomly assigned to the reactogenicity subset. Randomization at Visit 1 will also include assignment to vaccine or placebo group.

At each of the 3 injection visits, subjects assigned to the reactogenicity subset will receive a DC for also recording solicited reactions for 6 days after each injection and will receive instructions on how to complete the DC and how to return the completed DC to the site at the next planned visit. Otherwise, depending on randomization, the same steps will be followed for the main population or the immunogenicity subset.

SAEs and AEs That Are Related to Vaccination or That Led to Discontinuation:

At any time during the study, a subject who experiences an SAE or an AE must be followed if *either* of the following is true:

- The SAE or AE is considered by the Investigator to be related to vaccination, and is not resolved by the end of the subject's participation in the trial
- The subject has been discontinued from the trial because of the SAE or AE

Any such subject must be followed until the condition resolves, becomes stable, or becomes chronic.

5.1.4.4 Surveillance

Details of the surveillance for CDI are provided to the study sites in a document separate from this clinical protocol.

Surveillance for CDI throughout the study and stool collection

The site will be required to review each subject's medical history to complete certain eCRFs. To characterize severe, complicated CDI, details will be obtained from medical and hospital records and will include information, in part, concerning fever, elevated WBCs, ileus, pseudomembranous colitis, serum albumin measurement, abdominal distension, abdominal tenderness, and admission to the intensive care unit. This information will be obtained on an on-going basis throughout the study.

All subjects will be followed for efficacy (the primary occurrence or recurrence of a CDI) from Day 0 through the follow-up period for occurrence of repeated episodes of loose stools and provision of a stool sample. Some subjects may be followed for a shorter period of time if the trial is completed and the subject has not reached the maximum 3.0 year follow-up period.

For all subjects, there will be a weekly contact (telephone, home or clinic visit) for 4 weeks after enrollment (Day 0 through Day 30) or after discharge from a hospital, long-term care or rehabilitation facility to remind subjects to report repeated episodes of loose stools and provide stool samples, if applicable. Following the first 4 weeks, subjects will be contacted every 2 weeks for the duration of the study and follow-up period. The method of contact can vary (telephone, text message, email, and/or home visit); however, there must be subject contact via telephone or home visit once a month.

If a subject is hospitalized, or is admitted to a long-term care or rehabilitation facility, the subject will be contacted once a week until discharge or for a maximum of 3 months to monitor the subject for episodes of loose stools, for which a stool sample will be obtained and provided to the site. After discharge from the hospital or facility, the subjects will be contacted weekly for 4 weeks, then every 2 weeks thereafter for the remainder of the follow-up period (as described above).

During the follow-up period, for those subjects who experience repeated episodes of loose stools, the third or later stool sample will be collected in the collection container provided and the stool samples will be provided to the site. A repeat stool sample will not be collected if a subject has already been diagnosed locally as a clinical case, and the site has already frozen the stool sample for testing at the central laboratory. A repeat stool sample will be collected for recurrence if the subject again experiences loose stools episodes ≥ 14 days following a clinical cure (defined as ≤ 2 loose stools in 24 hours for 2 consecutive days following completion of 10 days of standard of care treatment for the initial CDI episode). Recurrence is defined as the development of a new episode of diarrhea (≥ 3 loose stools in ≤ 24 hours and loose stools lasting ≥ 24 hours, with a PCR-confirmed CDI-positive stool sample) that occurs ≥ 14 days after a clinical cure.

All contacts must be documented in the source documents. Admission and discharge dates will be recorded.

5.1.5 Planned Trial Calendar

The following dates are approximate. The actual dates may differ as, for example, the trial will not start until all the appropriate regulatory and ethical approvals have been obtained.

Planned trial period - FVFS (first visit, first subject) to LCLS (last contact, last subject): August 2013 – July 2021

Planned inclusion period - FVFS to FVLS (first visit, last subject): August 2013 – October 2019

Planned vaccination period: August 2013 – November 2019

Planned end of trial: July 2021

Planned date of final clinical trial report: December 2021

5.2 Enrollment and Retention of Trial Population

5.2.1 Recruitment Procedures

Each site will be responsible for devising a written recruitment plan for enrolling eligible subjects. This plan must specify how potential subjects will be identified, and how they will be informed of the study objectives and requirements. The site will ensure that any advertisements used to recruit subjects (letters, pamphlets, posters, etc.) are submitted to Sanofi Pasteur prior to submission to the IEC/IRB for approval.

5.2.2 Informed Consent Procedures

Informed consent is the process by which a subject voluntarily confirms his or her willingness to participate in a particular trial. Informed consent must be obtained before any study procedures are performed. The process is documented by means of a written, signed, and dated ICF.

In accordance with GCP, prior to signing and dating the consent form, the subject must be informed by appropriate study personnel about all aspects of the trial that are relevant to making the decision to participate, and must have sufficient time and opportunity to ask any questions.

According to local regulations, the presence of one or more witnesses will be required for all subjects, (e.g., in Latin America).

The actual ICF used at each center may differ, depending on local regulations and IEC/IRB requirements. However, all versions must contain the standard information found in the sample ICF provided by the Sponsor. Any change to the content of the ICF must be approved by the Sponsor and the IEC/IRB prior to the form being used.

If new information becomes available that may be relevant to the subject's willingness to continue participation in the trial, this will be communicated to him / her in a timely manner. Such information will be provided via a revised ICF or an addendum to the original ICF.

Informed consent forms will be provided in duplicate, or a photocopy will be made. The original will be kept by the Investigator, and the copy will be kept by the subject.

Documentation of the consent process should be recorded in the source documents.

5.2.3 Screening Criteria

There are no screening criteria other than the inclusion and exclusion criteria.

5.2.4 Inclusion Criteria

An individual must fulfill *all* of the following criteria in order to be eligible for trial enrollment:

1) Aged ≥ 50 years on the day of inclusion[a]
2) Informed consent form has been signed and dated
3) Able to attend all scheduled visits and to comply with all trial procedures
4) Covered by health insurance (only valid for specific countries)
5) Must fulfill at least 1 of the following criteria*

   Risk Stratum 1:

   - Has had at least 2 hospital stays, each lasting at least ≥ 24 hours, in the 12 months before enrollment
     and
   - Has received systemic (not topical) antibiotics in the 12 months before enrollment

   or

   Risk Stratum 2:

   - Is anticipated to have an in-patient hospitalization for a planned surgical procedure within 60 days of enrollment. The impending hospital stay is planned to be ≥ 72 hours for a surgery involving 1 of the following:
     - Kidney/bladder/urinary system
     - Musculoskeletal system
     - Respiratory system
     - Circulatory system
     - Central nervous system

* If an individual is either an in-patient in the hospital or had a previous hospitalization, the enrollment date must be at least 30 days from hospital discharge.

5.2.5 Exclusion Criteria

An individual fulfilling *any* of the following criteria is to be excluded from trial enrollment:

1) Subject is pregnant, or lactating, or of childbearing potential (to be considered of non-childbearing potential, a female must be post-menopausal for at least 1 year, surgically sterile,

[a] "≥ 50 years" means from the day of the 50th birthday

or using an effective method of contraception[a] or abstinence from at least 4 weeks prior to the first vaccination and until at least 4 weeks after the last vaccination)

2) Participation in the 4 weeks preceding the first trial vaccination or participation during the present trial period in another clinical trial investigating a vaccine, drug, medical device, or medical procedure
3) Receipt of any vaccine in the 4 weeks preceding the first trial vaccination except for influenza (seasonal or pandemic) and pneumococcal vaccines. This exception includes monovalent pandemic influenza vaccines and multivalent influenza vaccines.
4) Previous vaccination against *C. difficile* with either the trial vaccine, another vaccine, or monoclonal antibodies
5) Diarrhea on day of enrollment
6) Self-reported current or prior CDI episode
7) Anticipated or current receipt of kidney dialysis treatment
8) History of gastrointestinal surgery for gastrointestinal malignancy (*Note:* Colonoscopy, polypectomy, and appendectomy are not exclusion criteria.)
9) History of inflammatory bowel disease, irritable bowel syndrome (must include diarrhea as a symptom), colostomy, or small or large intestine bowel surgery where resection was performed
10) Receiving enteral feeding (e.g., nasogastric, gastrostomy, and jejunostomy tube feeding)
11) Known or suspected congenital or acquired immunodeficiency; or receipt of immunosuppressive therapy, such as anti-cancer chemotherapy or radiation therapy, within the preceding 6 months; or long-term systemic corticosteroid therapy (prednisone or equivalent for more than 2 consecutive weeks within the past 3 months)
12) Known systemic hypersensitivity to any of the vaccine components, or history of a life-threatening reaction to the vaccine used in the trial or to a vaccine containing any of the same substances[b]
13) Self-reported thrombocytopenia, contraindicating IM vaccination
14) Deprived of freedom by an administrative or court order, or in an emergency setting, or hospitalized involuntarily
15) Current alcohol abuse or drug addiction that might interfere with the ability to comply with trial procedures in the opinion of the Investigator

---

[a] Effective methods of contraception include oral contraception (pill), intrauterine device, diaphragm or condoms (only when used with spermicidal foam/gel/film/cream/suppository), hormonal implants, transdermal patch, or parenteral contraception. Abstinence is applicable when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of a trial, and withdrawal are not acceptable methods of contraception.

[b] The components of *C. difficile* Toxoid Vaccine are listed in Section 6.1.1.3 and in the Investigator's Brochure.

16) Chronic illness that, in the opinion of the investigator, is at a stage where it might interfere with trial conduct or completion[a]

17) Moderate or severe acute illness/infection (according to investigator judgment) on the day of vaccination or febrile illness (temperature ≥ 38.0°C [≥ 100.4°F]). A prospective subject should not be included in the study until the condition has resolved or the febrile event has subsided.

18) Identified as an Investigator or employee of the Investigator or study center with direct involvement in the proposed study, or identified as an immediate family member (i.e., parent, spouse, natural or adopted child) of the Investigator or employee with direct involvement in the proposed study

If required by local regulations, if the subject has a primary physician who is not the Investigator, the site should contact this physician to inform him / her of the subject's participation in the study. In addition, the site should ask this primary physician to verify exclusion criteria relating to previous therapies, such as receipt of blood products or previous vaccines.

5.2.6 Medical History

At the time of enrollment, subjects will be assessed for pre-existing conditions and illnesses, both past and ongoing. Any such conditions will be documented in the source document. Significant medical history (reported as diagnosis) including conditions for which the subject is or has been followed by a physician or conditions that could resume during the course of the study or lead to an SAE or to a repetitive outpatient care will be collected in the eCRF. The significant medical history section of the eCRF contains a core list of body systems and disorders to prompt comprehensive reporting.

Dates, medications (with the exception of those listed in Section 6.7), and body systems are not to be recorded, and the information collected will not be coded. Of note, antibiotics taken within 30 days of enrollment will be recorded with the start date, stop date, dose, and route, and will be coded. Its purpose is to assist in the later interpretation of safety data collected during the trial.

For each condition, the data collected will be limited to:

- Diagnosis (this is preferable to reporting signs and symptoms)
- Presence or absence of the condition at enrollment

5.2.7 Contraindications for Subsequent Vaccinations

5.2.7.1 Temporary Contraindications

Should a subject experience one of the conditions listed below, the Investigator will postpone further vaccination until the condition is resolved. Postponement must still be within the timeframe for vaccination indicated in the Table of Study Procedures.

[a] Chronic illness may include, but is not limited to, cardiac disorders, renal disorders, auto-immune disorders, or diabetes

- Febrile illness (temperature ≥ 38.0°C [≥ 100.4°F]) or moderate or severe acute illness / infection on the day of vaccination, according to Investigator judgment

5.2.7.2 Definitive Contraindications

Should a subject experience one of the conditions listed below, the Investigator will discontinue vaccination:

- Pregnancy, as indicated by a positive urine test
- An anaphylactic or other significant allergic reaction to the previous dose of vaccine
- Any SAE related to the administration of vaccine, not only anaphylactic or significant allergic reaction to the previous dose, will preclude additional vaccination of the subject.

Subjects will not be withdrawn due to contraindication but will be followed up for safety and possibly immunogenicity assessment.

5.2.8 Other Conditions for Withdrawal than Definitive Contraindications

Subjects will be informed that they have the right to withdraw from the trial at any time. A subject may be withdrawn from the study:

- At the discretion of the Investigator or Sponsor due to safety concerns (withdrawal) without the subject's permission
- At the request of the subject (dropout)

The following will result in automatic withdrawal or exclusion of a subject from the study:

- Significant noncompliance with the protocol, based on the Investigator's judgment

The reason for a withdrawal or dropout should be clearly documented in the source documents and on the eCRF.

The Investigator must determine whether voluntary withdrawal is due to safety concerns (in which case, the reason for discontinuation will be noted as "SAE" or "other AE" as appropriate) or for another reason.

5.2.9 Lost to Follow-up Procedures

In the case of subjects who fail to return for a follow-up examination or do not respond to surveillance contacts, documented reasonable effort (i.e., documented telephone calls, certified mail, or home visit) should be undertaken to locate or recall them, or at least to determine their health status while fully respecting their rights. These efforts should be documented in the eCRF and in the source documents.

5.2.10 Classification of Subjects Who Discontinue the Trial

For any subject who discontinues the trial prior to completion, the most serious reason for early termination will be checked in the eCRF. Reasons will be listed from the most serious to the least serious as follows:

- **Serious adverse event:** To be used when a subject drops out of or is withdrawn from the study by the Investigator because of the occurrence of an SAE, as defined in Section 9.2.3.1.
- **Other adverse event:** To be used when a subject drops out of or is withdrawn from the study by the Investigator because of the occurrence of an AE other than an SAE, as defined in Section 9.2.3.1.
- **Non-compliance with protocol:** To be used when the Investigator withdraws a subject from the study because of failure to follow protocol guidelines (e.g., not attending visits, not being available for telephone calls, not providing blood samples). This termination category may also be used if it is retrospectively discovered that a subject did not fulfill the eligibility criteria. The Investigator will provide a comment as to the specific cause of non-compliance.
- **Lost to follow-up:** To be used when the Investigator withdraws a subject from the study because of failure to establish contact, as outlined in Section 5.2.9. The Investigator will provide documentation that contact was attempted (i.e., return of unsigned certified letter receipt; not located during the home visit).
- **Voluntary withdrawal not due to an adverse event:** To be used when a subject drops out of the study for any reason other than those listed above.

5.2.11 Follow-up of Discontinuations

The site should complete all scheduled safety follow-ups with any subject who has prematurely terminated the trial because of an SAE, other type of AE, non-compliance with the protocol, or loss of eligibility, including definite contraindications.

For subjects where the reason for early termination was lost to follow-up or if the subject withdrew informed consent and specified that they do not want to be contacted again and it is documented in the source document, the site will not attempt to obtain further safety information.

When early termination is due to an AE or SAE, loss of eligibility, non-compliance with the protocol, or definite contraindications, the subject will be contacted.

For subjects where the reason for early termination is voluntary withdrawal, the site will attempt to contact them for the 6-month follow-up except if they specified that they do not want to be contacted again and it is documented in the source document.

Subjects who discontinue the study will be followed for a period of 6 months after the last injection.

5.2.12 Follow-up and Reporting of Pregnancies

Pregnancy is an exclusion criterion for enrollment in this study, but a subject could potentially become pregnant during her participation. In case of pregnancy and if at least 1 dose of the study

vaccine has been administered, the subject will not be discontinued from the trial and will be followed for safety assessment (and may be followed for immunogenicity assessment, if applicable). However, no additional vaccination will be administered.

All pregnancy cases should be reported if they occurred during the study and during the 6-month follow-up period (i.e. through Day 210). To report the pregnancy case, the Investigator must fill out a Presence of Pregnancy Form in the eCRF and submit it to the Sponsor immediately after identifying a pregnancy case. Study staff must then maintain contact with the subject to obtain information about the outcome—i.e., details about the delivery and the newborn, or about pregnancy termination—and must update the Pregnancy Reporting Form, this time marking the "Follow-up" checkbox. This information should be provided to the Sponsor within 1 month of delivery.

Pregnancy itself is not considered an AE, but any complications during pregnancy are to be considered as AEs, and in some cases could be considered SAEs. Spontaneous abortions, fetal death, stillbirth, and congenital anomalies reported in the baby are always considered as SAEs, and the information should be provided to the Global Pharmacovigilance (GPV) Department regardless of when the SAE occurs (e.g., even after the end of the trial).

5.3 Safety Emergency Call

If, as per the Investigator's judgment, a subject experiences a medical emergency, the Investigator may contact the Sponsor's RMO for advice on trial related medical question or problem. If the RMO is not available, then the Investigator may contact a Call Center – available 24 hours a day, 7 days a week – that will forward all safety emergency calls to the appropriate primary or back-up Sanofi Pasteur contact, as needed. The toll-free contact information for the Call Center will be provided in the Operating Guidelines.

This process does not replace the need to report an SAE. The investigator is still required to follow the protocol defined process for reporting SAEs to GPV (Please refer to Section 10).

In case of emergency code-breaking, the Investigator is required to follow the code-breaking procedures described in Section 6.4.

5.4 Modification of the Trial and Protocol

Any amendments to this trial plan and protocol must be discussed with and approved by the Sponsor. If agreement is reached concerning the need for an amendment, it will be produced in writing by the Sponsor, and the amended version of the protocol will replace the earlier version. All substantial amendments e.g. that affect the conduct of the trial or the safety of subjects, require IEC / IRB approval, and must also be forwarded to regulatory authorities according to local regulations.

An administrative amendment to a protocol is one that modifies some administrative or logistical aspect of the trial but does not affect its design or objectives or have an impact on the subjects' safety. Regulatory authorities may be notified of administrative changes and will provide approval according to local regulations.

The Investigator is responsible for ensuring that changes to an approved trial, during the period for which IEC/IRB approval has already been given, are not initiated without IEC/IRB review and approval, except to eliminate apparent immediate hazards to subjects.

5.5 Interruption of the Trial

The trial may be discontinued if new data about the investigational product resulting from this or any other trials become available; or for administrative reasons; or on advice of the Sponsor, the Investigators, and / or the IECs/IRBs. If the trial is prematurely terminated or suspended, the Sponsor shall promptly inform the Investigators, the regulatory authorities, the IECs/IRBs, and IDMC of the reason for termination or suspension, as specified by the applicable regulatory requirements.

The Investigator shall promptly inform the trial subjects and assure appropriate therapy and / or follow-up for them.

The IDMC will periodically assess the progress of the clinical trial and recommend to Sanofi Pasteur whether to continue, modify, or stop it. The IDMC will be responsible for the evaluation of the safety of *C. difficile* Toxoid Vaccine, for any decisions for unblinding the subject codes, and for making recommendations to the Sponsor on a medical and ethical basis. Also the IDMC will be the only group (in addition to the unblinded statistician) with access to the unblinded analyses at the 4 interim analyses. At each of the interim analyses, the IDMC will review the unblinded data and advise to either continue the trial as planned or to stop the trial (for efficacy or for futility) using the statistical testing as primary guidance for their recommendation.

The trial may also be halted for efficacy or futility at the time of the second, third, and fourth interim analyses. Please refer to Section 5.1.1 for a description of these halting rules.

6 Vaccines Administered

Subjects will receive either the investigational product *C. difficile* Toxoid Vaccine or placebo.

6.1 Identity of the Investigational Product

6.1.1 Identity of Trial Product

C. difficile Toxoid Vaccine is a formalin-inactivated vaccine developed for the prevention of primary CDI. The vaccine is a highly purified preparation of toxoids A and B. Toxin A and toxin B were purified from cultures of *C. difficile*, inactivated, and mixed. The vaccine is presented as a lyophilized preparation in a vial and is reconstituted with diluent. The 0.5 mL dose is administered IM.

6.1.1.1 Composition

[redacted]

[redacted]

6.1.1.2 Preparation and Administration

Specific instructions for preparation of dosing solutions will be provided by the Sponsor in the Operating Guidelines. Briefly, the unblinded vaccine preparer will take the pre-filled syringe containing diluent, add it to the vial containing lyophilized vaccine, and then draw up the reconstituted vaccine for administration. According to local practice, the vaccine administrator may also be unblinded and may be the same person as the vaccine preparer. However, since the vaccine preparation (which is cloudy) can be distinguished from the placebo (which is clear), the individual responsible for the vaccine preparation and/or administration will be unblinded and all personnel involved in safety assessments will be blinded to group assignment. The 0.5 mL dose will be administered IM.

Prior to administration, all study products must be inspected visually for cracks, broken seals, correct label content (see Section 6.3.1), and extraneous particulate matter and / or discoloration, whenever solution and container permit. If any of these conditions exists, the vaccine must not be administered. In this case, IVRS/IWRS will be called for a replacement dose, and the event is to be reported to the Sponsor.

The doses on Days 0 and 7 will be injected into alternate arms, if possible (i.e., the vaccination nurse will use the opposite arm each time). If it is not possible to administer vaccine in alternate arms, then the same arm may be used for consecutive vaccinations. In this case, the vaccine administrator should make every attempt to administer the consecutive vaccination in an area distinct from that of the previous vaccination. The injection on Day 30 may be in either arm.

Subjects must be kept under observation for 30 minutes by staff blinded to group assignment after vaccination to ensure their safety, and any reactions during this period will be documented in the eCRF. Appropriate medical equipment and emergency medications, including epinephrine (1:1000), must be available on site in the event of an anaphylactic or other immediate allergic reaction.

6.1.1.3 Dose Selection and Timing

Each 0.5 mL dose of reconstituted vaccine contains 100 µg of *C. difficile* toxoid A and toxoid B plus 400 µg aluminum (see also Section 6.1.1.1). Subjects in the vaccine group of the study will receive 1 injection of vaccine on each of Days 0, 7, and 30.

6.1.2 Identity of Control Product

6.1.2.1 Composition

The placebo product is 0.9% normal saline.

6.1.2.2 Preparation and Administration

Specific instructions for preparation of dosing solutions will be provided by the Sponsor in the Operating Guidelines. Briefly, normal saline will be provided in a vial, and will be drawn up into the empty syringe by the vaccine preparer for administration. According to local practice, the vaccine administrator may also be unblinded and may be the same person as the vaccine preparer. Since the placebo (saline solution, which is clear) can be distinguished from the vaccine preparation (which is cloudy), the individual responsible for the vaccine preparation and/or administration will be unblinded and all personnel involved in safety assessments will be blinded to group assignment. The 0.5 mL dose will be administered IM.

See also Section 6.1.1.2.

6.1.2.3 Dose Selection and Timing

Subjects in the placebo group of the study will receive 1 injection on each of Days 0, 7, and 30.

6.2 Identity of Other Products

Not applicable.

6.3 Product Logistics

6.3.1 Labeling and Packaging

The lyophilized *C. difficile* Toxoid Vaccine will be provided in a vial. The diluent for reconstitution will be provided in a syringe.

Each study vaccine kit will include 1 vial of lyophilized *C. difficile* Toxoid Vaccine and 1 syringe of diluent, i.e., materials for 1 vaccination. Each subject will be assigned 3 separate product kits. The placebo kit will provide saline solution in a vial and an empty syringe. At each of the 3 injection visits, a unique product kit number will be assigned by calling into the IVRS/IWRS system.

The units and the boxes will bear the requirements specific to study H-030-014.

For some countries, where acceptable and in accordance with local regulations, primary labels are in English with information in local language on the carton; for other countries primary and secondary labels are in local language in accordance with local regulations.

6.3.2 Product Shipment, Storage, and Accountability

6.3.2.1 Product Shipment

The Clinical Logistics Coordinator or designee will contact the Investigator or a designee in order to determine the dates and times of delivery of products.

Each vaccine shipment will include a temperature-monitoring device to verify maintenance of the cold chain during transit. On delivery of the product to the site, the person in charge of product receipt will follow the instructions given in the Operating Guidelines, including checking that the cold chain was maintained during shipment (i.e., verification of the temperature recorders). If there is an indication that the cold chain was broken, this person should immediately quarantine the product, alert the Sanofi Pasteur representative, and request authorization from Sanofi Pasteur to use the product.

6.3.2.2 Product Storage

The Investigator will be personally responsible for product management or will designate a staff member to assume this responsibility.

At the site, products must be kept in a secure place with restricted access. Vaccines will be stored in a refrigerator at a temperature ranging from +2°C to +8°C. The vaccines must not be frozen. The temperature must be monitored and documented (according to the information given in the Operating Guidelines) for the entire time that the vaccine is at the trial site. In case of accidental freezing or disruption of the cold chain, vaccines must not be administered and must be quarantined, and the Investigator or authorized designee should contact the Sanofi Pasteur representative for further instructions.

6.3.2.3 Product Accountability

The person in charge of product management at the site will maintain records of product delivery to the trial site, product inventory at the site, the dose(s) given to each subject, and the disposal of or return to the Sponsor of unused doses.

The necessary information on the product labels is to be entered into the source document and the eCRF.

The Sponsor's monitoring staff will verify the trial site's product accountability records against the record of administered doses in the eCRFs and the communication from the IVRS/IWRS (if applicable).

In case of any expected or potential shortage of product during the trial, the Investigator or an authorized designee should alert the Sanofi Pasteur representative as soon as possible, so that a shipment of extra doses can be arranged.

6.3.3 Replacement Doses

If a replacement dose is required (e.g., because the syringe broke or particulate matter was observed in the syringe), the site personnel will call IVRS/IWRS. Selection of another study test kit of placebo or vaccine from the stock of product for administration will be based on the randomization scheme. Additional instructional details are provided in the Operating Guidelines.

6.3.4 Disposal of Unused Products

Unused or wasted investigational product will either be returned to Sanofi Pasteur or a delegated facility, or upon written Sanofi Pasteur approval, disposed of at the site, after reconciliation. Instructions will be provided in the Operating Guidelines.

Product accountability will be verified throughout the trial period.

6.3.5 Recall of Products

If the Sponsor makes a decision to launch a retrieval procedure, the Investigators will be informed of what needs to be done.

6.4 Blinding and Code-breaking Procedures

This is an observer-blind study. The injections will be prepared in a separate room to ensure that the blind is maintained.

The IVRS/IWRS vendor will be responsible for providing the treatment group identification to be received by the enrolled subject. In order to minimize the potential risk of unintentional bias when assessing safety an observer-blind procedure will be followed. The subject, the Investigator, and study staff members who collect the safety data and laboratory personnel who analyze the blood and stool samples will all be blinded to the group assignment. The individual responsible for preparing the vaccine may also administer the vaccine and will not be authorized to collect any safety/serology data. If the individual who administers the vaccine is a different individual from the preparer, the individual who administers the vaccine is also unblinded and will not be authorized to collect any safety/serology data. In addition, the unblinded staff or authorized designee will have to ensure that the documents on randomization are stored in a secure place where only he/she has access. In order to maintain the study blind, the clinical personnel blinded to group assignment should not be present during product administration.

The code may be broken:

- by the Investigator only in the event of an SAE and if identification of the vaccine received could influence the treatment of the SAE. Code-breaking should be limited, as far as possible, to the subject(s) experiencing the SAE.

The blind can be broken by the Investigator or a sub-investigator (medical doctor only) by calling the IVRS/IWRS, as explained in the code-breaking procedures described in the Operating Guidelines. Once the emergency has been addressed by the site, the Investigator must notify the Sanofi Pasteur Responsible Medical Officer if a subject's code was broken. All contact attempts prior to unblinding are to be documented in the source documents.

A request that the code be broken may be made:

- by the IDMC if needed to facilitate their assessment of safety

In all cases, the IEC / IRB must be notified of the code-breaking. All documentation pertaining to the event must be retained in the site's study records and in the Sanofi Pasteur files. Any

intentional or unintentional code-breaking must be reported, documented, and explained, and the name of the person who requested it must be provided to the Sponsor.

The code will be broken in case of any reported death or 2 life threatening SAEs where the Investigator's causality assessment is "Related" to the investigational product. The PSO will request that the subject's treatment group be unblinded. In this case, the code will be broken only for the subject(s) in question. The information resulting from code-breaking (i.e., the subject's vaccine or group assignment) will not be communicated to either the Investigator or any other clinical team member of the Sponsor.

Health authorities may request code-breaking in the case of an SAE as described in ICH E2A. In this case, the code will be broken only for the subject(s) in question. The information resulting from code-breaking (i.e., the subject's vaccine or group assignment) will not be communicated to either the Investigator or the Sponsor. This code will be broken by the PSO and will not be communicated to the clinical team or the Investigators.

Any intentional or unintentional code-breaking must be reported, documented, and explained, and the name of the person who requested it must be provided to the Sponsor.

In case of interim analysis, the blinded Clinical Data Manager will inform the IDMC that the targeted milestone of the total number of CDI case has been observed for any of the specified interims. The independent unblinded statistician will retrieve the blinded data from the Data Manager, merge the blinded data with the randomization code, evaluate the unblinded data (the numbers of observed clinical CDI endpoints will be computed for each randomization group [case splits] and estimates of VE will be computed) and send the results to the IDMC.

No one other than the independent unblinded statistician and the IDMC will be allowed to have access to the unblinded data or see the results.

6.5 Randomization and Allocation Procedures

The Sponsor or designee will supply a centralized, risk-stratified, computer-generated randomization code. The computer-generated randomization code will assign subjects to 1 of 2 groups. Subjects may also be assigned to the immunogenicity subset (10% of total enrollment) or reactogenicity subset (20% of total enrollment).

Stratification will be based on the geographical region and the 2 major inclusion strata.

There are 4 geographical regions: US and Canada, Latin America, Europe, and South East Asia. The 2 major inclusion strata will be defined as follows:

Risk Stratum 1:

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

and

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

or

Risk Stratum 2:

- Is anticipated to have an in-patient hospitalization for a planned surgical procedure within 60 days of enrollment and the impending hospital stay is planned to be ≥ 72 hours (see Section 5.2.4, Inclusion Criteria).

This stratification, together with region-blocks randomization (each region will have a block of randomization spaces with the maximum allowable enrollment cap), and a fixed block size of 3, will ensure the planned 2:1 balance between vaccine and placebo groups during the entire course of study enrollment. The relative numbers of subjects enrolled into each stratum will be monitored continuously during the trial. If the fraction enrolled to any 1 stratum drops below 30%, then the study team will review the operational approaches to recruitment and recommend changes to the approach that will increase the relative rate of enrollment to the smaller group. The randomization scheme will be designed to maintain the 2:1 ratio within each of the participating regions.

Each subject who meets the eligibility criteria and signs an informed consent form will be randomly assigned to 1 of 2 groups according to a 2:1 ratio via an IVRS/IWRS. Subjects will also be assigned to the immunogenicity subset (10% of total enrollment) or reactogenicity subset (20% of total enrollment). Site staff will call the IVRS/IWRS, enter identification and security information, and confirm a minimal amount of data in response to IVRS/IWRS prompts. The IVRS/IWRS will then state the group assignment, whether the subject is randomized to the immunogenicity or the reactogenicity subset, and have the caller confirm this. Subject numbers will be recorded on the eCRFs. The full detailed procedures for randomization are described in the Operating Guidelines.

Subject numbers will be 8 digits long, with a 3-digit center identifier and a 5-digit subject identifier. For example, Subject 001-00001 is the first subject enrolled in center number 1. Part of the 5-digit number will be unique identifiers of subjects in each of the subsets.

Subject identification numbers should not be reassigned for any reason. The Quality Assurance Department at Sanofi Pasteur will hold the randomization codes in a secured location.

6.6 Treatment Compliance

The following measures will ensure that the vaccine doses administered comply with those planned, and that any non-compliance is documented so that it can be accounted for in the data analyses:

- All vaccinations will be administered by qualified trial personnel

- The person in charge of product management at the site will maintain accountability records of product delivery to the trial site, product inventory at the site, doses given to each subject, and the disposal of unused or wasted doses.

6.7 Concomitant Medications or Other Therapies

At the time of enrollment, ongoing medications including other therapies, e.g., blood products, should be recorded in the source document as well as new medications prescribed for new medical conditions/AEs during trial participation. In particular, antibiotics taken within the 30 days of enrollment will be recorded, with the start and stop dates.

Documentation in the eCRF of concomitant medication will be limited to specific categories of medication of interest beginning on the day of vaccination (Day 0). This may include medications of interest that were started prior to the day of vaccination.

Reportable medications will be collected in the eCRF from the day of vaccination (except for antibiotics for which collection starts 30 days prior to enrollment) to the end of the solicited and unsolicited follow-up period in general (e.g., 30-day safety follow-up) as they may impact the response to the vaccination and impact the consistency of the information collected on concomitant medications at any vaccination. Following Day 60, through the end of the long-term follow-up period (up to 3.0 years), reportable medications in Category 2 and Category 3 will be collected in the eCRF.

The “reportable” medications are distributed according to 3 categories. These are:

- Category 1: antipyretics, analgesics, non steroidal anti-inflammatory drugs (NSAIDs)
- Category 2: non-study vaccines
- Category 3: antibiotics , antibiotics administered specifically for CDI treatment, probiotics, CDI treatment by donor stool transfer, anti-diarrhea medications

The information reported in the eCRF for each reported medication will be limited to:

- Trade name
- Given as treatment or as prophylaxis
- Medication category
- Start and stop dates
- Dosage and administration route for antibiotics when used for treatment of CDI

Homeopathic medication will not be recorded. Topical treatment will not be recorded.

Medication given in response to an AE will be captured in the “Action Taken” column of the AE only. No details will be recorded in the concomitant medication module of the eCRF unless the medication received belongs to one of the prelisted categories. Only medications in the prelisted categories will be coded.

7 Management of Samples

Blood samples for potential assessment of antibody responses will be collected at Visits 1 and 4 for all subjects. Additionally blood will be collected for approximately 1650 subjects in the immunogenicity subset in accordance with the Tables of Study Procedures.

Stool samples will be collected by the subjects according to protocol specifications, sent to the clinical site for storage, and then shipped to a designated laboratory for testing.

7.1 Sample Collection

7.1.1 Blood Samples

For all subjects 20 mL of blood will be collected in tubes provided by or recommended by the Sponsor. See the Tables of Study Procedures for the visits at which blood will be drawn.

Immediately prior to the blood draw, the staff member performing the procedure will verify the subject's identity. Labels will be pre-printed with the subject's number; the label will be attached to the tube. If blood sampling occurs on the same day as a vaccination, the blood is to be taken from the limb opposite to the one that will be used for vaccination.

If a subject refuses the blood sampling, the subject will be discontinued from the study due to non-compliance with the protocol.

7.1.2 Stool Samples

The Sponsor will provide stool collection kits that will be given to each subject, and subjects will be asked to provide a stool sample from the third episode (or later) of loose stools in the event of the occurrence of multiple episodes within a 24 hour period. Subjects will be instructed to collect stool samples throughout the study. Stool samples will be placed immediately into the storage container provided. The subjects will be instructed to complete the MA-LS with stool collection information and to contact the clinical site to alert them that there is a stool sample ready for pick-up. Stool sample pick-up may be by courier or site staff. Alternatively, the subject may deliver the stool sample to the clinical site. The stool sample must be stored in the cooled shipping container until it is picked up or delivered to the clinical site within 72 hours of collection. It is important for the stool samples that are determined by the site to meet the clinical definition of CDI be tested by PCR.

The site will divide the sample into 2 or 3 aliquots: 1 aliquot will be sent to the central laboratory (for testing for study), and 1 aliquot will be kept as a retention sample. The third aliquot is optional and can be used for testing at the local site (for patient treatment, if part of standard of care within that region). The address of the central laboratory for *C. difficile* toxin testing by PCR and storage will be provided in the Operating Guidelines. If the test results are positive, a sample will be shipped from the central laboratory to another laboratory for REA typing. If local tests are performed, copies of the test results will be maintained with the source documents. Any tests for *C. difficile* or other enteropathogens will be documented by the site in the eCRF. The Investigator

will treat the subject as per standard of care. Details of this process will be provided in the Operating Guidelines.

7.1.3 Urine Samples

Urine will be collected according to site procedures for pregnancy testing. Urine will be collected from women of child-bearing potential before vaccination on Days 0, 7, and 30.

7.2 Sample Preparation

7.2.1 Blood Samples

Detailed instructions on how to prepare blood samples for assessment of antibody response are contained in the Operating Guidelines provided to the site. An overview of the procedures is provided here.

Following the blood draw, the tubes are to be left undisturbed, positioned vertically and not shaken, for a minimum of one hour and a maximum of 2 hours in order to allow the blood to clot. Samples can be stored at room temperature for up to 2 hours; beyond 2 hours, they must be refrigerated at a temperature of +2°C to +8°C to a maximum of 24 hours from collection.

The samples are then centrifuged, and the serum is transferred to the appropriate number of aliquoting tubes. These tubes are pre-labeled with adhesive labels that identify the study code, the subject's number, and the visit number.

The subject's identification number, the date of sampling, the number of aliquots obtained, the date and time of preparation, and the subject's consent for future use of his/her samples are to be specified on a sample identification list and recorded in the source document. Space is provided on this list for comments on the quality of samples.

7.2.2 Stool Samples

Detailed instructions on how to handle stool samples will be provided in the Operating Guidelines provided to the site. An overview of the procedure is provided here.

Before being delivered or sent to the site, stool samples will be stored in the cooled shipping container; the sample must be delivered to the site within 72 hours of collection. At the site, stool samples are to be kept in a freezer in which the temperature is set and maintained at -20°C or below. The temperature will be monitored and documented on the appropriate form during the entire trial. If it rises above -10°C for any period of time, the Clinical Logistics Coordinator must be notified. Storage and shipping will be conducted according to information provided in the Operating Guidelines.

7.2.3 Urine Samples

Samples will be analyzed immediately after collection.

7.3 Sample Storage and Shipment

7.3.1 Serum Samples

During storage, serum tubes are to be kept in a freezer whose temperature is set and maintained at -20°C or below. The temperature will be monitored and documented on the appropriate form during the entire trial. If it rises above -10°C for any period of time, the Clinical Logistics Coordinator must be notified. Further details are provided in the Operating Guidelines.

Shipments to the laboratories will be made only after appropriate monitoring, and following notification of the Clinical Logistics Coordinator or delegate. Sera will be shipped frozen, using dry ice to maintain them in a frozen state, in the packaging container provided by the carrier. Again, temperatures will be monitored. Shipments must be compliant with the International Air Transport Association (IATA) 602 regulations.

Samples will be shipped to Global Clinical Immunology (GCI) at Sanofi Pasteur. The address is
Global Clinical Immunology, Building 53
Sanofi Pasteur Inc.
Discovery Drive
Swiftwater, PA 18370-0187

[redacted] [redacted]

7.3.2 Stool Samples

Detailed instructions for the labeling and shipping of stool samples will be covered in the Operating Guidelines.

7.3.3 Urine samples

Urine samples (for pregnancy testing) will be analyzed immediately upon collection and will not be stored.

7.4 Future Use of Stored Serum and Stool Samples for Research

Any unused part of the serum or stool samples will be securely stored at the Sanofi Pasteur serology laboratory (GCI) in Swiftwater, PA, USA, or at the CRO for at least 5 years after the last license approval in the relevant market areas has been obtained for the vaccine being tested.

In accordance with country specific Health Authorities, serum or stool samples may not be allowed for future use.

Where future use is allowed, and if approved by the IRB/IEC, subjects will be asked to indicate in the ICF whether they will permit the future use of any unused stored serum or stool samples for other tests. If they refuse permission, the samples will not be used for any testing other than that directly related to this study. If they agree to this use, they will not be paid for giving permission. (Anonymity of samples will be ensured.) The aim of any possible future research is unknown today, and may not be related to this particular study. It may be to improve the knowledge of

vaccines or infectious diseases, or to improve laboratory methods. Genetic tests will never be performed on these samples without individual informed consent.

8 Clinical Supplies

Sanofi Pasteur will supply the trial sites with protocols, ICFs, eCRFs, eSAE reporting forms, diary cards, memory aids, and other trial documents, as well as with the following trial materials: all study vaccines or placebo (each product kit includes a syringe and a vial), needles, blood collection tubes, stool collection kits, storage container for cold storage of stool samples, cryotubes, cryotube storage boxes, cryotube labels, temperature recorders (if required), shipping containers, rulers, pregnancy tests, and digital thermometers.

The means for performing electronic data capture (EDC) will be defined by Sanofi Pasteur. If a computer is provided by Sanofi Pasteur, it will be retrieved at the end of the trial.

The Investigator will supply all phlebotomy, vaccination, and centrifugation equipment, including biohazard and/or safety supplies, as well as self-addressed stamped envelopes for the return of diary cards. The biohazard and safety supplies include needles and syringes, examination gloves, laboratory coats, sharps disposal containers, and absorbent countertop paper. The site will ensure that all biohazard wastes are autoclaved and disposed of in accordance with local practices. The Investigator will also supply appropriate space in a temperature-monitored refrigerator for the storage of the products and for the blood samples, and appropriate space in a temperature-monitored freezer maintained -20°C or below for serum and stool aliquots.

In the event that additional supplies are required, study staff must contact Sanofi Pasteur, indicating the quantity required. Contact information is provided in the Operating Guidelines.

9 Endpoints and Assessment Methods

9.1 Primary Endpoints and Assessment Methods

The primary efficacy objective is:

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

9.1.1 Efficacy Endpoints

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

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

  Notes:

  - Timing of 24-hour period will start from the first episode of loose stools

  - Loose stool is defined as type 6 (fluffy pieces with ragged edges, a mushy stool) or type 7 (watery, no solid pieces; entirely liquid) according to the Bristol Stool Chart (38).

and

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

or

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

9.1.2 Efficacy Assessment Methods

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

9.2 Secondary Endpoints and Assessment Methods

9.2.1 Efficacy

The secondary efficacy objectives are:

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

9.2.1.1 Efficacy Endpoints

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

9.2.1.2 Efficacy Assessment Methods

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

9.2.2 Immunogenicity

The secondary immunogenicity objectives are:

- To describe the immunogenicity to toxin A and toxin B:
  - in the subset (1650) of subjects and in subjects with CDI (250) at Day 0 and Day 60 (± 14 days)

  and

  - in the subset (1650) of subjects at Day 14 (+3 days), Day 30 (-3 days to +7 days), and every 6 months up to 3.0 years (± 14 days) after the third injection

9.2.2.1 Immunogenicity Endpoints

- Serum antibody concentrations against toxins A and B, measured by ELISA
- Serum antibody titers against toxins A and B, measured by TNA
- Exploratory assays may be performed

9.2.2.2 Immunogenicity Assessment Methods

ELISA testing:

Serum will be tested by ELISA for IgG antibodies to *C. difficile* toxin A and toxin B to generate primary immunogenicity data. Additional evaluations of antibody responses may be performed to

further characterize the immune response to vaccination with *C. difficile* Toxoid Vaccine, which may include other serum immunoglobulins to *C. difficile* toxin A and toxin B.

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

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

Toxin neutralization assay:

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

The test utilizes the accumulation of acid metabolites in closed culture wells as an indication of normal cell respiration. In cells exposed to toxin, metabolism and CO_2 production is reduced; consequently, the pH rises to 7.4 or higher as indicated by the phenol red pH indicator in the cell culture medium. At this pH, the medium appears red. Control cells, or cells exposed to toxin which has been neutralized by antibody, however, metabolize and produce CO_2 in normal amounts; as a result, the pH is maintained at 7.0 or below. At this pH, the medium appears yellow. Therefore, *C. difficile* toxin neutralizing antibodies correlate with the ability of the serum to neutralize the metabolic effects of *C. difficile* toxin on cells as evidenced by their ability to maintain a pH of 7.0 or lower. The color change of the media can be measured at 562-630 nm by a plate reader to further calculate the antitoxin neutralizing antibody titer at 50% inhibition of the *C. difficile* toxin-mediated cytotoxicity.

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

9.2.3 Safety

The secondary safety objective is:

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

9.2.3.1 Safety Definitions

The following definitions are taken from the ICH E2A Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting.

Adverse Event:

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding, for example), symptom or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Therefore an AE may be:

- A new illness
- The worsening of a concomitant illness
- An effect of vaccination, including the comparator
- A combination of the above

All AEs include serious and non-serious AEs.

Surgical procedures are not AEs; they are the action taken to treat a medical condition. It is the condition leading to the action taken that is the AE (if it occurs during the trial period).

Pre-existing medical conditions are not to be reported as AEs. However, if a pre-existing condition worsens in frequency or intensity, or if in the assessment of the treating physician there is a change in its clinical significance, this change should be reported as an AE (exacerbation). This applies equally to recurring episodes of pre-existing conditions (e.g., asthma) if the frequency or intensity increases post-vaccination.

Serious Adverse Event:

Serious and *severe* are not synonymous. The term *severe* is often used to describe the intensity of a specific event as corresponding to Grade 3. This is not the same as *serious* which is based on patient / event outcome or action criteria usually associated with events that pose a threat to a patient's life or functioning. Seriousness, not severity, serves as a guide for defining regulatory reporting obligations.

An SAE is any untoward medical occurrence that at any dose (including overdose):

- Results in death
- Is life-threatening[a]
- Requires inpatient hospitalization or prolongation of existing hospitalization[b]

---

[a] The term "life-threatening" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.

[b] All medical events leading to hospitalizations will be recorded and reported as Serious Adverse Events, with the exception of: hospitalization planned before inclusion into the study or out-patient treatment with no hospitalization.

- Results in persistent or significant disability / incapacity[a]
- Is a congenital anomaly / birth defect
- Is an important medical event[b]

Adverse Reaction:

All noxious and unintended responses to a medicinal product related to any dose should be considered AR.

(The phrase "responses to a medicinal product" means that a causal relationship between a medicinal product and an AE is at least a reasonable possibility)

Unexpected Adverse Reaction:

An unexpected adverse reaction (UAR) is an AR, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved investigational medicinal product).

The following additional definitions are used by Sanofi Pasteur:

Solicited Reaction:

A solicited reaction is an AE that is prelisted in the eCRF**.** The assessment of these AEs post-vaccination is mandatory. A solicited reaction is defined by a combination of:

- Symptom and
- Onset post-vaccination

e.g., injection site pain between D0 and D6 post-vaccination, or headache between D0 and D6.

A solicited reaction is therefore an AR observed and reported under the conditions (symptom and onset) prelisted (i.e., solicited) in the CRF and considered as related to vaccination.

Unsolicited AE / AR:

An unsolicited AE is an observed AE that does not fulfill the conditions prelisted in the CRF in terms of symptom and / or onset post-vaccination, i.e., excluding solicited reactions, e.g., if headache between D0 and D6 is a solicited reaction (i.e., prelisted in the CRF), then a headache

---

[a] "Persistent or significant disability or incapacity" means that there is a substantial disruption of a person's ability to carry out normal life functions.

[b] Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the health of the subject or may require intervention to prevent one of the other outcomes listed in the definition above. These should also usually be considered serious. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse, new onset diabetes or autoimmune disease.

starting on D6 is a solicited reaction, whereas headache starting on D8 post-vaccination is an unsolicited AE.

An unsolicited non-serious AE is an unsolicited AE excluding SAEs.

Injection Site Reaction:

An injection site reaction[a] is an AR at and around the injection site. Injection site reactions are commonly inflammatory reactions.

Systemic AE:

Systemic AEs are all AEs that are not injection site reactions. They therefore include systemic manifestations such as headache, fever, as well as localized or topical manifestations that are not associated with the vaccination site, e.g., erythema that is localized but that is not at the injection site.

9.2.3.2 Safety Endpoints

The secondary endpoints for the evaluation of safety are:

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

[a] All injection site AEs are considered to be related to vaccination and are therefore all *injection site reactions*.

9.2.3.3 Safety Assessment Methods

9.2.3.3.1 Immediate Post-vaccination Surveillance Period

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

- Any unsolicited systemic AE observed to occur during the first 30 minutes post-vaccination will also be recorded on the eCRF, i.e., as immediate unsolicited systemic AE
- Solicited and unsolicited injection site reactions and solicited systemic reactions will not be actively solicited during the 30 minute-period. If they occur within 30 minutes of vaccination, they will be recorded and analyzed as starting on the day of vaccination
- Any SAE occurred during the first 30 minutes post-vaccination will be reported in the same way as any other SAE and to the Sponsor, according to the procedures described in Section 10.

9.2.3.3.2 Reactogenicity (Solicited Reactions From Day 0 to Day 6 After Each Vaccination)

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

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

Table 9.1 and Table 9.2 present, respectively, the injection site reactions and systemic reactions that are prelisted in the diary cards and CRF, together with the intensity scales.

The doses on Days 0 and 7 will be injected into alternate arms, if possible (i.e., the vaccination nurse will use the opposite arm each time). If the vaccine is administered in alternate arms, then the injection site reactions after each dose can be independently assessed. If it is not possible to administer vaccine in alternate arms, then the same arm may be used for consecutive vaccinations. The Day 30 dose may be injected into either arm.

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

Table 9.1: Solicited injection site reactions: terminology, definitions, and intensity scales

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

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

Table 9.2: Solicited systemic reactions: terminology, definitions, and intensity scales

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

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

Important notes for the accurate assessment of temperature:

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

9.2.3.3.3 Unsolicited Adverse Events From Day 0 to Day 30 After Each Vaccination

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

- Start and stop dates[a]
- Intensity of the event:
- For measurable unsolicited AEs that are part of the list of solicited reactions, the size of the AE as well as the temperature for fever will be collected and analyzed based on the corresponding scale used for solicited reactions (see Table 9.1 and Table 9.2).
- Other unsolicited non-serious AEs will be classified according to the following intensity scale:

  Grade 1: No interference with activity

  Grade 2: Some interference with activity

  Grade 3: Significant; prevents daily activity

- Action taken for each AE, if any (e.g., medication)
- Whether the AE led to discontinuation

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

9.2.3.3.4 Serious Adverse Events

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

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

Any SAE occurring at any time during the trial will be reported by the Investigator through the EDC system and according to the completion guidelines provided by the Sponsor. All information concerning the SAE is to be reported, either as part of the initial reporting or during follow-up reporting if relevant information became available later (e.g., outcome, medical history, results of investigations, copy of hospitalization reports). The Investigator will assess the causal relationship between the SAE and the investigational product as either "Not related" or "Related", as described in Section 9.2.3.3.5.

See Section 10 for further details on SAE reporting.

9.2.3.3.5 Assessment of Causality

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

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

0: None

1: Medication (self-medication with an existing prescription or over-the-counter medication)

2: Health care provider contact (no new medication prescribed)

3: Health care provider contact and prescription of a new medication (health care provider instructed subject to take a new medication, either an over-the-counter medication or one requiring a written prescription)

4: Hospitalization (inpatient)

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

0: None

1: Medication (self-medication with an existing prescription or over-the-counter medication)

2: Health care provider contact (no new medication prescribed)

3: Health care provider contact and prescription of a new medication (health care provider instructed subject to take a new medication, either an over-the-counter medication or one requiring a written prescription)

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

[a] ICH Guidelines, Clinical Safety Data Management E2A

0: Not related – The AE is clearly / most probably caused by other etiologies such as subject's underlying condition, therapeutic intervention, or concomitant therapy; or the delay between vaccination and the onset of the AE is incompatible with a causal relationship; or the AE started before the first vaccination (screening phase, if applicable)

1: Related – There is a "reasonable possibility" that the AE was caused by the vaccination, meaning that there is evidence or arguments to suggest a causal relationship

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

9.3 [redacted]

- [redacted]

10 Reporting of Serious Adverse Events

In order to comply with current regulations on SAE reporting to health authorities, the Investigator must document all SAEs regardless of causal relationship, and notify the Sponsor and the local Clinical Research Associate (CRA) within the notification timelines stated in the following sections. The Investigator will give access and provide the Sponsor and the local CRA with all necessary information to allow the Sponsor to conduct a detailed analysis of the safety of the investigational product. It is the responsibility of the Investigator to request all necessary documentation (e.g., medical records, discharge summary, autopsy) in order to provide comprehensive safety information. All relevant information must then be transcribed into the e-SAE Form.

Information on all SAEs regardless of causal relationship will be collected and assessed throughout the trial, from inclusion through the end of the surveillance period.

Note: Some subjects will be enrolled in Risk Stratum 2, which specifies anticipation of in-patient hospitalization for a planned surgery. This hospitalization that occurs as part of the enrollment criteria will not be considered nor reported as an SAE.

10.1 Initial Reporting by the Investigator

SAEs occurring during a subject's participation in the trial or experiment must be reported within 24 hours of the Investigator becoming aware of the SAE to the Sponsor's GPE Department and to the CRA. Every SAE must be reported, even if the Investigator considers that it is not related to the vaccine. The SAE form must be signed by a licensed physician (MD or DO) for whom the task is listed on the Study Task Delegation and Signature List after each update to the Form.

The Investigator must complete the "e-SAE Form" in the electronic data capture (EDC) application. After validation, an e-mail alert will automatically be sent to the GPE mailbox, the CRA. This message will include the country, the study code, the subject number, whether the report is an initial or a follow-up report, the diagnosis and / or symptoms, the seriousness criteria, the relationship, if related, and the outcome, if fatal.

If the EDC system is unavailable, the site must notify the Sponsor using the paper version of the SAE Reporting Form, as follows:

The Investigator must complete the SAE Reporting Form, check off the "Initial Reporting Form" box, and send it to the Sponsor by one of the following means:

- By fax, to the following number: [redacted]
- In PDF format to the following e-mail address, using a method of transmission that includes password protection: [redacted]

When the system becomes available, the Investigator must transcribe the information from the paper version of the eSAE Form into the EDC system.

10.2 Follow-up Reporting by the Investigator

The e-SAE Form completed initially must be updated within 24 hours after the Investigator has become aware of any new relevant information concerning the SAE (e.g., outcome, precise description of medical history, results of the investigation). After validation, an e-mail alert will be sent automatically to the GPE Department and to the CRA. All relevant information must be included directly in the e-SAE form. Copies of documents (e.g., medical records, discharge summary, autopsy) may be requested by the GPE Department.

The anonymity of the subject must always be respected when forwarding this information.

10.3 Reporting of SAEs Occurring After a Subject Has Completed the Study

Any SAE that occurs after a subject has completed the study but that is likely to be related to the product or to the experiment must also be reported as soon as possible. In such a case, the reporting procedure to be followed is identical to that described in Section 10.1.

10.4 Assessment of Causality

The causal relationship between the SAE and the product will first be evaluated by the Investigator, using the following definitions:

> **0 - Not related**: The AE is clearly / most probably caused by other etiologies such as an underlying condition, therapeutic intervention, or concomitant therapy; or the delay between vaccination and the onset of the SAE is incompatible with a causal relationship; or the SAE started before the first vaccination (screening phase, if applicable).
>
> **1 - Related**: There is a "reasonable possibility" that the SAE was caused by the vaccination, meaning that there is evidence or arguments to suggest a causal relationship.
>
> *(ICH Guidelines, Clinical Safety Data Management E2A)*

Following this, the Sponsor's Product Safety Officer (PSO) will also assess the causal relationship to the product, based on the available information and current medical knowledge.

The decision to modify or discontinue the trial, or to break individual or all trial codes, may be made after mutual agreement between the Sponsor and the Investigators.

10.5 IDMC

An IDMC consisting of at least 5 independent physicians and at least 1 biostatistician external to Sanofi Pasteur with relevant subject matter expertise will be in place during this study.

Safety

The IDMC members will receive for review all related SAE reports within 3 working days (72 hours) of notification to the Sponsor. A report indicating the SAE review, recommendations for follow-up or further information, and an opinion on enrollment will be provided to the Sponsor's RMO by the IDMC within 7 working days of receipt of their notification of the SAE.

The IDMC may unblind a subject's treatment assignment as part of their review and may call for a full meeting of the IDMC at any time to review any SAEs of concern. Additional specific details regarding the composition, organization and operation of the IDMC are contained within the IDMC charter.

The IDMC will be advised of the safety data for study participants throughout the follow-up period.

Collection of SAE cases will continue throughout the trial. For analysis purposes, the primary safety evaluation period will include SAEs reported up to 6 months after the last dose of study vaccine. SAEs with an outcome of death or non-fatal SAEs leading to hospitalization will also be submitted for review and adjudication by a blinded external Adjudication Committee, as per IDMC request. The procedures governing the function of this Committee are outlined in a separate Manual of Operations included in the IDMC Charter. The results of the review and adjudication will also be provided to the IDMC and form part of the IDMC safety data review.

Interim Analyses for Efficacy and Futility

The IDMC will be the only group with access to the unblinded analyses at the 4 interim analyses. At each of the interim analyses, the IDMC will review the unblinded data and advise to either continue the trial as planned or to stop the trial (for efficacy or for futility) using the aforementioned statistical testing as primary guidance for their recommendation. In addition to the statistical guidelines for early stopping with a finding of efficacy, a recommendation of early stopping for efficacy by the IDMC will also require a minimum of a 12-month median duration. In case of an early stopping, each subject will be followed for safety for at least 6 months after the last injection. Under the trial design assumptions, at least a 12-month median duration of follow-up is expected to be obtained at the time of the third interim analysis (i.e., the second interim analysis for efficacy). However, if the projected duration of follow-up falls somewhat short of the 12-month requirement, the timing of that interim analysis will be delayed until a median of 12 months of follow-up for all subjects is achieved, in order to ensure that this operational criterion is met before the formal statistical test for efficacy.

10.6 Reporting SAEs to Health Authorities and IECs / IRBs

The Sponsor will inform the relevant health authorities of any reportable SAEs according to the local regulatory requirements. Reporting to the health authorities will be according to the Sponsor's standard operating procedures.

The Sponsor's RMO or designee will notify the Investigators in writing of the occurrence of any reportable SAEs. The Investigators / Sponsor, according to local regulations, will be responsible for informing the IECs or IRBs that reviewed the trial protocol.

11 Data Collection and Management

11.1 Data Collection and eCRF Completion

Individual safety diary cards, specifically designed for this trial by the Sponsor and provided to the study sites, will be given to study participants for the recording of daily safety information as described in Section 9.2.3.3.

For subjects in the reactogenicity subset, these diary cards will include prelisted terms and intensity scales (see Table 9.1 and Table 9.2 as well as areas for free text to capture additional safety information or other relevant details). Subjects will also be provided with rulers for measuring the size of injection site reactions, and with standard digital thermometers for measuring daily temperatures. To ensure consistency of reporting, the study sites will instruct subjects on how to correctly use these tools.

The 6-month follow-up will be done by interviewing subjects either during a visit or over the telephone using a questionnaire to capture SAEs and AEs of particular interest, if applicable. A memory aid will be provided to the subjects at the preceding trial visit to help them record information on events occurring between this visit and the 6-month follow-up, and throughout the surveillance period.

Relevant information will be transcribed into the eCRF. Any SAEs captured during this 6-month follow-up period and throughout the surveillance period will be reported and followed-up as per the normal process for reporting SAEs.

At specified intervals, the Investigator or an authorized designee will interview the subjects to collect the information recorded in the diary card, and will attempt to clarify anything that is incomplete or unclear. All clinical trial information gathered by the study site will be reported electronically by the Investigator or authorized designee using a web-based CRF. (Any information that was not documented in the diary card will first be captured in the source document and then reported electronically.) The eCRF has been designed specifically for this trial under the responsibility of the Sponsor, using a validated Electronic Records/Electronic Signature-compliant platform (21 CFR Part 11).

To ensure the correct and consistent completion of the eCRFs, the Sponsor or authorized representative will provide all necessary tools, instructions, and training to all site staff involved in data entry prior to study start. Additional instructional documents such as training manuals and completion guidelines will be provided to assist with data entry during the course of the trial.

Upon completion of training, each user requiring access to the EDC system will be issued a unique username and password. In the event of a change in trial personnel, each newly assigned individual will receive a unique username and password; the username and password of a previous user may not be reissued. If any trial personnel leave the study, the Investigator is responsible for informing the Sponsor immediately so that their access is deactivated. An audit trail will be initiated in the EDC system at the time of the first data entry in order to track all modifications and to ensure database integrity.

The Investigator is responsible for the timeliness, completeness, and accuracy of the information in the eCRFs; must provide explanations for all missing information; and must sign the eCRF using an e-signature.

11.2 Data Management

Management of Clinical Data

Data generated during the trial will be managed following 2 different processes:

- Clinical data, defined as all data reported in the eCRF, review data received from the blinded external Adjudication Committee, and laboratory data will be handled by the Sponsor's Clinical Data Management (CDM) platform or authorized representative.
- Data pertaining to SAEs, which are reported by the Investigator on the eSAE Forms or SAE Reporting Forms, will be handled by the Sponsor's GPE Department.

During the trial, clinical data reported in the eCRFs will be integrated into the clinical database under the responsibility of the Sanofi Pasteur CDM platform. Data monitoring at the sites and quality control in the form of computerized logic and / or consistency checks will be systematically applied in order to detect errors or omissions. In addition, data reviews may be performed several times by the Sponsor's staff in the course of the trial. Any questions pertaining to the reported clinical data will be submitted to the investigator for resolution using the EDC system. Each step of this process will be monitored through the implementation of individual passwords to maintain appropriate database access and to ensure database integrity.

The validation of the immunogenicity data will be performed at the laboratory level following the laboratory's procedures. Information from the laboratory will be checked for consistency before integration into the clinical database.

After integration of all corrections in the complete set of data, and after the SAE information available from CDM and the GPE Department has been reconciled, the database will be released for statistical analysis.

SAE Data Management

During the trial, data pertaining to SAEs reported on e-SAE Forms will be integrated into the Sponsor's centralized GPE database.

Upon receipt of an e-SAE Form, the data will be entered into the GPE database after a duplicate check. Each SAE case will be assigned a case identification number. All cases will be reviewed and assessed by the PSO. Each case is reviewed, locked, and approved in the GPE database before being reported to the relevant authorities as necessary. Follow-up information concerning a locked and approved case will be entered into the GPE database, and a new version of the case will be created.

The information pertaining to SAEs in the GPE database will be reconciled with that in the clinical database.

11.3 Data Review

A blind review of the data is anticipated through the data review process led by Data Management before database lock.

12 Statistical Methods and Determination of Sample Size

This study will enroll approximately 16,500 subjects.

The study is designed as an event-driven, group sequential protocol with 4 interim analyses at 50, 100, 135, 167 clinical endpoints (i.e., PCR-confirmed CDI episodes), and a final analysis when 250 clinical endpoints are reached. The first interim will assess futility only at observing 50 CDI cases. The second interim analysis will assess futility and efficacy at observing 100 CDI cases. The third interim analysis will assess efficacy and occur when 135 clinical endpoints are observed. The fourth interim and final analyses will be performed at 167 and 250 CDI cases, respectively.

Analyses of trial futility (non-efficacy) will be performed at the first 2 interim analyses. The study may be stopped if either of those analyses provides robust and compelling evidence that meaningful levels of vaccine efficacy (VE) will not be demonstrated. [redacted]

Analyses of efficacy will be performed at the second, third, and fourth interim analyses, as well as, the final analysis, with the total one-sided type 1 error rate at 0.025 (See Table 5.1). The nominal significance levels for the 3 efficacy interim analyses are 0.01, 0.0035, and 0.0032, respectively, as determined by the corresponding proportion of information assessed at each interim analysis (number of new cases at each interim / 250 * 0.025). The remaining alpha (0.0083) after the interim analyses will be used at the final analysis. Based on the trial simulations, if the true VE is 60%, there is an estimated 45% probability to reject the primary null hypothesis at the second interim (first interim for efficacy).

[redacted]

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

The trial will be monitored by an IDMC who will be the only group with access to the unblinded analyses at the 4 interim analyses. At each of the interim analyses, the IDMC will review the unblinded data and advise to either continue the trial as planned or to stop the trial (for efficacy or for futility) using the aforementioned statistical testing and operational criteria as primary guidance for their recommendation.

Process for the final analyses:

Following the decision of stopping the study by the IDMC at any of the interim analyses or at the final, the data will be locked and ready for analyses. The randomization code will be released. Data in the Clinical Data Management database will be analyzed under the responsibility of the Biostatistics Platform of the Sponsor, with SAS software, at least version 9.2 (SAS Institute, Cary, North Carolina, USA).

12.1 Statistical Methods

The following sections describe the primary, secondary, and exploratory analyses, further details on these analyses and sub-analyses can be found in the Statistical Analysis Plan.

12.1.1 Hypotheses and Statistical Methods for Primary Objective

12.1.1.1 Efficacy

12.1.1.1.1 Primary Hypothesis for Efficacy

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

H_0: VE $\leq$ 15% *vs.* H_A: VE > 15%

where VE=1-R_V/R_P and R_V and R_P are the rates of having a CDI endpoint during follow-up in the vaccinated and placebo groups, respectively.

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

12.1.1.1.2 Statistical Method for the Primary Hypothesis for Efficacy

At the second, third, and fourth interim analyses and at the final analysis, the primary hypothesis of VE will be evaluated. These tests will be based on computing point and interval estimates of VE using standard Poisson-based methods for estimating event rates and assessing whether the resulting CIs cover the null hypothesis value for VE, with an overall one-sided type 1 error rate of 0.025. In the case of efficacy testing, the nominal significance levels for the 3 efficacy interim analyses are 0.01, 0.0035, and 0.0032, respectively. The remaining alpha (0.0083) after the interim analyses will be used for the final analysis. The primary null hypothesis will be rejected if the CI computed at the nominal sizes describe above fail to cover the null VE value of 15%. The analyses will be performed on the modified intent-to-treat (MITT) population. We note that if interim analyses are performed based on total numbers of cases that are not precisely the same as the aforementioned target milestones, the nominal significance levels at which the interim analyses will be performed and those of the following tests will be adjusted accordingly.

Notes:

- Age is an important factor; VE will be calculated in different age categories for each of the study groups.
- Antibiotics received within the 30 days prior to enrollment are also factors of interest; VE will be calculated for subjects who received antibiotics within 30 days prior to enrollment in each of the study groups.

12.1.1.2 Futility

12.1.1.2.1 Primary Hypothesis for Futility

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

Formally, the following hypothesis will be used:

H_0: VE $\geq$ 46% *vs.* H_A: VE < 46%

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

12.1.1.2.2 Statistical Method for the Primary Hypothesis for Futility

The study will be stopped for futility (non-efficacy) at either of the first or second interim analyses if the upper limit of the 2-sided 95% CI for VE computed using standard Poisson-based estimates of relative endpoint rates amongst the vaccine and placebo groups is less than the alternative hypothesis value for VE of 46%.

12.1.1.3 Endpoints for the Primary Hypothesis

Endpoints are the symptomatic PCR-confirmed primary CDI cases, defined as:

- Presence of both of the following clinical symptoms:
  - $\geq$ 3 loose stools in $\leq$ 24-hours
  - loose stools lasting $\geq$ 24 hours

  Notes:

  - Timing of 24-hour period will start from the first episode of loose stools

  - Loose stool is defined as type 6 (fluffy pieces with ragged edges, a mushy stool) or type 7 (watery, no solid pieces; entirely liquid) according to the Bristol Stool Chart (39).

and

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

or

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

12.1.2 Hypotheses and Statistical Methods for Secondary Objectives

12.1.2.1 Secondary Hypotheses for Efficacy

1) The same as the primary hypothesis as in Section 12.1.1.1, the population used in the analyses is per-protocol population.
2) The same as the primary hypothesis as in Section 12.1.1.1, the population used in the analyses is subjects who received at least 2 doses of the vaccine.
3) The relative risk of PCR-confirmed CDI cases is not constant over time since enrollment (H_0: RR≠1 *vs.* H_1: RR=1). This will be tested using a 2-sided alpha of 0.05.
4) The secondary statistical null hypothesis is that there is no reduction in the risk of severe PCR-confirmed cases of CDI among the subjects receiving the vaccine relative to subjects receiving placebo. Formally this hypothesis will test:

   H_0: VE ≤ 0% *vs.* H_A: VE > 0%

   where $VE=1-R_V/R_P$ and R_V and R_P are the rates of having a severe CDI endpoint during follow-up in the vaccinated and placebo groups, respectively.
5) For the secondary hypothesis, the proportion of subjects in the 3 loose stool categories (frequency of subjects with the maximum loose stools per day in the vaccine and those in the placebo group during the CDI episode will be categorized into 3 categories; 3-5, 6-10 and >10 loose stools) in the vaccine group are equal to those in the placebo group. Formally this hypothesis will test:

   H_0: $\pi_{iv} = \pi_{ip}$ *vs.* H_A: $\pi_{iv} \neq \pi_{ip}$, i = 1, 2 and 3 is any one of the 3 loose stool categories; 3-5, 6-10 and > 10 loose stools, where π_{iv} and π_{ip} are the proportion of subjects in the i^{th} category in the vaccination group and the corresponding proportion in the placebo group, respectively. This hypothesis will be tested using a 2-sided alpha of 0.05.
6) For the secondary hypothesis, there is no reduction in CDI episode/illness duration in subjects who are symptomatic primary PCR-confirmed CDI cases. Formally this hypothesis will test:

   H_0: $\tau_v \geq \tau_p$ *vs.* H_A: $\tau_v < \tau_p$

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

12.1.2.2 Statistical Methods for the Secondary Hypotheses for Efficacy

Applicable to 1) and 2) above:

The same statistical methods used in the analyses of primary objectives will be used for 1) and 2) of the secondary objectives. However, the size of the associated tests and CIs computed for secondary analyses will not be formally adjusted for multiplicity because these analyses will be performed only once at the conclusion of the trial. The secondary hypotheses will be performed separately using 2 populations:

- Per-protocol subjects
- Subjects who completed at least 2 injections

For the secondary hypothesis 3), durability of the VE: a time-dependent model for the relative risk of PCR-confirmed CDI endpoints will be fit to the trial data to assess trends in VE as a function of time since enrollment. This model will be formally compared with the standard constant relative risk model to test (at 2-sided 0.05 level) whether any observed departures from constancy are statistically significant.

The same statistical methods used in the analyses of primary objectives will be used for 4) of the secondary hypothesis. However, the size of the associated test and CIs computed for this analyses will not be formally adjusted for multiplicity because this analyses will be performed only once at the conclusion of the trial. This secondary hypothesis will be testing (H_0: VE $\leq$ 0% *vs.* H_A: VE > 0%). Where VE=1-R_V/R_P and R_V and R_P are the true rates of having a severe CDI case during the follow-up period in the vaccine and placebo groups, respectively.

For the secondary hypothesis 5), the frequency of subjects with the maximum loose stools per day in the vaccine and those in the placebo group during the CDI episode will be categorized into 3 categories; 3-5, 6-10 and >10 loose stools. Comparison of the proportions of the subject in the 3 categories in the vaccine and placebo groups will be made using Fisher exact test, using 2-sided alpha of 0.05. The following hypothesis will be tested: H_0: $\pi_{iv} = \pi_{ip}$ vs H_A: $\pi_{iv} \neq \pi_{ip}$, i = 1, 2 and 3 is any one of the 3 loose stool categories; 3-5, 6-10 and >10, where π_{iv} and π_{ip} are the proportion of subjects in the i^{th} category in the vaccination group and the corresponding proportion in the placebo group, respectively. If the test is significant, the assumption of equality of the effect of the two vaccination groups will be rejected.

For the secondary hypothesis 6), comparison of the CDI episode/illness duration in vaccine and placebo groups will be made using Log Rank Test. The duration of each CDI episode/illness in the vaccine and the placebo groups will be collected and their distributions will be compared using the Log Rank Test using a one-sided alpha of 0.05. The following hypothesis will be tested: H_0: $\tau_v \geq \tau_p$ *vs.* H_A: $\tau_v < \tau_p$ where τ_v and τ_p are the CDI episode/illness duration in the vaccine and placebo group, respectively, in the symptomatic PCR-confirmed CDI cases. If there is a significant reduction in CDI episode/illness duration in the vaccine group, the null hypothesis will be rejected.

12.1.2.3 Endpoints for the Secondary Hypotheses for Efficacy

Applicable to 1) and 2) above, the same endpoints as Section 12.1.1.3.

3) Number and date of PCR-confirmed CDI endpoints

4) CDI endpoints as in the primary hypothesis (Section 12.1.1.3) defined as severe

5) The maximum number of loose stools per day associated with a symptomatic PCR-confirmed primary CDI case

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

12.1.3 Secondary Objectives for Immunogenicity

- To describe the immunogenicity to toxin A and toxin B:
  - in the subset (1650) of subjects and in subjects with CDI (250) at Day 0 and Day 60 (± 14 days)

  and

  - in the subset (1650) of subjects at Day 14 (+3 days), Day 30 (-3 days to +7 days), and every 6 months up to 3.0 years after the third injection

12.1.3.1 Statistical Method for the Secondary Objectives for Immunogenicity

The geometric mean concentration (GMC) of the serum anti-toxin IgG concentrations by ELISA and geometric mean titers (GMTs) anti-toxin neutralization assay (TNA) titers to *C. difficile* toxins A and B up to 3.0 years after the third injection will be calculated as well as their 95% CI assuming the concentration is log normally distributed. The GMCs will be calculated at Days 0 and 60, also at each of the planned the additional bleeds.

12.1.3.2 Endpoints for the Secondary Objectives for Immunogenicity

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

12.1.4 Secondary Objectives for Safety

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

12.1.4.1 Statistical Method for the Secondary Objective for Safety

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

- Unsolicited AEs occurring within 30 minutes of each injection
- Solicited injection site reactions occurring within 6 days after the day of each injection (Day 0 to Day 6) for subjects in the reactogenicity subset
- Solicited systemic reactions occurring within 6 days after the day of each injection (Day 0 to Day 6) for subjects in the reactogenicity subset
- Unsolicited AEs occurring within 30 days after each injection by SOC and preferred term
- All SAEs that occur through 6 months after the last injection
- SAEs that are considered related to vaccination and/or study procedures from Day 210 through the end of the follow-up period

12.1.4.2 Endpoints for the Secondary Objectives for Safety

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

12.1.5 [redacted]

[redacted]

[redacted]

12.2 Analysis Sets

12.2.1 Safety Analysis Set

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

12.2.2 Modified Intent-to-Treat Analysis Set

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

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

12.2.3 Per-Protocol Analysis Set

12.2.3.1 Per-Protocol Efficacy Analysis Set

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

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

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

12.2.3.2 Protocol Immunogenicity Analyses Set:

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

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

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

12.2.4 Full Analysis Set

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

12.2.5 Other Analysis Sets

12.2.6 Populations Used in Analyses

- Primary Hypotheses:

Subjects with PCR-confirmed CDI case in the MITT will be used in the primary efficacy hypothesis.

- Secondary Hypotheses

Efficacy:

Subjects with PCR-confirmed CDI case in the PP efficacy analyses set will be used in the secondary efficacy hypothesis 1.

Subjects with PCR-confirmed CDI case who received at least 2 doses in the MITT will be used in the secondary efficacy hypothesis 2.

Subjects with PCR-confirmed CDI case in the MITT will be used in the secondary relative risk hypothesis 3.

Subjects with PCR-confirmed severe CDI case in the MITT will be used in the secondary efficacy hypothesis 4.

Immunogenicity:

All subjects in the FAS who participated in the subset and subjects with PCR-confirmed CDI case will be included in the immunogenicity analyses.

The same immunogenicity analyses will be performed using the PP immunogenicity subjects.

Safety:

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

[redacted]

12.3 Handling of Missing Data and Outliers

12.3.1 Safety

Missing data will not be replaced. Unsolicited AEs and SAEs with missing causality will be considered as related to vaccination, and will be identified as "unsolicited reactions". Missing intensity will remain missing and will not be imputed, unless the preferred term matches a measurable solicited reaction defined in the protocol (e.g.: injection site erythema and injection site swelling). (Note for SAEs: Intensity is not recorded.)

Further details will be described in the SAP.

12.3.2 Immunogenicity

For the calculation of GMTs and seroprotection, any pre-vaccination or post-vaccination titer reported as < LLOQ (lower limit of quantitation) will be converted to a value of 0.5 LLOQ.

Missing data will not be imputed. No test or search for outliers will be performed.

12.3.3 Efficacy

Missing CDI occurrence data will not be imputed. No test or search for outliers will be performed.

12.4 Interim / Preliminary Analysis

Details of the interim analysis can be found in the introduction of Section 12, Section 12.1.1.1.2, and Section 12.1.1.2.2.

12.5 Determination of Sample Size and Power Calculation

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

C. difficile Vaccine Group: n = 11,000

Placebo Group: n = 5500

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

Risk Stratum 1:

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

and

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

or

Risk Stratum 2:

- Is anticipated to have an in-patient hospitalization for a planned surgical procedure within 60 days of enrollment and the impending hospital stay is planned to be ≥ 72 hours (see Section 5.2.4, Inclusion Criteria).

This stratification, together with blocked randomization, will ensure the planned 2:1 balance between vaccine and placebo groups during the entire course of study enrollment. The relative numbers of subjects enrolled into each stratum will be monitored continuously during the trial. If the fraction enrolled to any 1 stratum drops below 30%, then the study team will review the operational approaches to recruitment and recommend changes to the approach that will increase the relative rate of enrollment to the smaller group.

Power of the Trial

The trial is designed to distinguish a null hypothesis of VE ≤ 15% from an alternative hypothesis value of VE > 46% with 90% power and an overall type 1 error rate of 0.025 (1-sided). Total sample size will be 16,500, randomized in a 2:1 ratio for the *C. difficile* vaccine and placebo groups. With 250 CDI cases, sample size of 16,500 subjects, and follow up for a maximum of up to 3 years (+ 30 days) after the third injection for each subject, the power calculations was based on the following assumptions:

1) An observed VE = 46%* and the lower bound of the 95% CI > 15%
2) Overall size of test for H_0 = 0.025 (1-sided)
3) Loss to follow-up: = 10% per year
4) Endpoint Rate (placebo)=1.5% per year

5) Enrollment Rate = 150 per week

**Note*: Because of small imperfections in the sensitivity and specificity of PCR, an observed 15% reduction in PCR-confirmed cases of CDI corresponds to an estimated true VE of 20%. An observed 46% is corresponding to an estimated true 60%.

If the trial does not stop for either futility or efficacy at an interim analysis, and CDI case accrual is not anticipated to achieve 250 cases by the completion of follow-up of the last enrolled participant, the trial will stop at approximately 180 cases. This number of endpoints provides at least 90% power for an observed vaccine efficacy of 50%.

13 Ethical and Legal Issues and Investigator / Sponsor Responsibilities

13.1 Ethical Conduct of the Trial / Good Clinical Practice

The conduct of this trial will be consistent with the standards established by the Declaration of Helsinki and compliant with the ICH guidelines for good clinical practice (GCP) as well as with all local and/or national regulations and directives.

13.2 Source Data and Source Documents

"Source data" are the data contained in source documents. Source documents are original documents or certified copies, and include, but are not limited to, diary cards, medical and hospital records, screening logs, informed consent / assent forms, telephone contact logs, and worksheets. The purpose of trial source documents is to document the existence of subjects and to substantiate the integrity of the trial data collected. Investigators must maintain source documents so that they are accurate, complete, legible, and up to date.

For missing or discrepant data on a diary card, the study coordinator will obtain verbal clarification from the subject, enter the response into the source document, and transfer the information to the eCRF.

The subject screening and enrollment log should list all individuals contacted by the Investigators to participate in the trial, regardless of the outcome.

[redacted]

The Investigator must print[a] any electronic records on an ongoing basis, sign and date them immediately after creation, and keep the printouts on file as source documents that can be verified by the Sponsor or an inspector against the electronic records. Any later changes of an electronic

[a] Unless the electronic medical records are managed by validated computerized systems that are compliant with US 21 CFR Part 11, in which case they are acceptable on their own.

record require the record to be re-printed, dated (with an indication of the date of change), and signed. Such records must also be kept together with the original printed copy.

13.3 Confidentiality of Data and Access to Subject Records

At the Study Site

Prior to initiation of the trial, the Investigator will sign a fully executed confidentiality agreement with Sanofi Pasteur.

Sanofi Pasteur personnel (or designates), the IECs / IRBs, and regulatory agencies, including the FDA, require direct access to all study records, and will treat these documents in a confidential manner.

At Other Non-Study Sites

If allowed by local regulations, the Sponsor and / or Investigator will need to have access to any medical records of the subjects. In the best interest of the subjects and the trial, the Principal Investigator will be responsible to ensure access to any medical records of the subjects.

13.4 Monitoring, Auditing, and Archiving

13.4.1 Monitoring

Before the start of the trial (i.e., before the inclusion of the first subject in the first center), the Investigators and the Sponsor's monitoring staff or a representative will meet at the site-initiation visit to discuss the trial protocol and the detailed trial procedures. Emphasis will be placed on inclusion and exclusion criteria, visit timing, safety procedures, informed consent procedures, SAE reporting procedures, eCRF completion, and the handling of samples and products.

The Sponsor's monitoring staff or a representative will ensure and document that all material to be used during the trial has been received at the site; and that the study investigator team and local monitoring staff have been properly informed about the trial, GCP and regulatory requirements, and the Sponsor's procedures. Specific training sessions for the study investigator team and the CRAs on these topics may be performed as necessary, and should be documented.

The following instruction manuals will be provided: the CRF Completion Guidelines for entering data into the eCRF, and the Operating Guidelines for detailed trial procedures such as the product management and sample-handling procedures.

After the start of the trial, the Sponsor's monitoring staff or a representative will be in regular contact with the investigational team through telephone calls and regular follow-up visits. The Investigator or delegate must be available for these visits, and must allow the monitoring staff direct access to subject medical files and eCRFs. During these visits, the monitoring staff will:

- Evaluate the quality of the trial progress (adherence to protocol and any study-specific guidelines, quality of data collection and document completion, signature of consent forms, occurrence of SAEs, sample and product management, cold-chain monitoring, archiving)
- Source-verify completed eCRFs and any corresponding answered queries

- Determine the number of complete or ongoing issues identified at monitoring visits (e.g., protocol violations, SAEs). Any identified problems will be discussed with the Investigator, and corrective or preventive actions will be determined, as appropriate.
- After all protocol procedures have been completed and the data have been entered into the eCRF, the Investigator or delegate must still be available to answer any queries forwarded by the Sponsor. All data-related queries must be completed prior to database lock.

At the end of the trial, a close-out visit will be performed to ensure that:

- The center has all the documents necessary for archiving
- All samples have been shipped to the appropriate laboratories
- All unused materials and products have been either destroyed or returned to the Sponsor

13.4.2 Audits and Inspections

A quality assurance (QA) audit may be performed at any time by the Sponsor's QA Department or by independent auditors to verify that the trial has been conducted according to the protocol, GCP and ICH requirements, and other applicable regulations. An inspection may be conducted by regulatory authorities. The Investigator must allow direct access to trial documents during these inspections and audits.

13.4.3 Archiving

After database lock, the Sponsor will provide each study site with a copy of all CRF data in a PDF format.

The Investigator must keep all trial documents for the duration indicated in the Clinical Trial Agreement or longer if required by local regulation after the completion or discontinuation of the trial, whatever the nature of the investigational center (private practice, hospital, institution). The Investigator will inform Sanofi Pasteur of any address change.

The Sponsor, or subsequent owner, will retain all documentation pertaining to the trial for the lifetime of the product. Archived data may be held on microfiche or electronic records, provided that a back-up exists and that a hard copy can be obtained if required. The protocol, documentation, approvals, and all other documents related to the trial, including certificates attesting that satisfactory audit and inspection procedures have been carried out, will be kept by the Sponsor in the Trial Master File (TMF). Data on AEs are included in the TMF. All data and documents will be made available if requested by relevant authorities.

13.5 Financial Contract and [redacted]

A Clinical Trial Agreement will be signed by all the parties involved in the trial's performance, if relevant. [redacted]

[redacted]

14 References List

1 Centers for Disease Control and Prevention, Food and Drug Administration, National Institutes of Health. Report of the Emerging Clostridial Disease Workshop, May 11, 2006: Summary of Proceedings. Atlanta, GA: Department of Health and Human Services;2006.

2 Bartlett JG, Gerding DN. Clinical recognition and diagnosis of Clostridium difficile infection. Clin Infect Dis. 2008;46 Suppl 1:S12-8.:S12-S18.

3 Rupnik M, Wilcox MH, Gerding DN. Clostridium difficile infection: new developments in epidemiology and pathogenesis. Nat Rev Microbiol. 2009;7:526-536.

4 Murphy CR, Avery TR, Dubberke ER, Huang SS. Frequent hospital readmissions for Clostridium difficile Infection and the Impact of Estimates of Hospital-Associated C. difficile Burden. Infect Control Hosp Epidemiology. 2012;33:20-28.

5 Landau SB, Monath TP. Physicians identify clostridium difficile colitis as a serious problem in need of new immune therapies. Gastrointestinal Endoscopy 1997;45(4).

6 Warny M, Kelly CP. Pathogenesis of Clostridium difficile toxins. In: Hecht GA, editor. Microbial pathogens and the intestinal epithelial cell. American Society for Microbiology Press; 2003. Chapter 27, p. 503-524.

7 Kelly CP and Thomas LaMont J. Clostridium difficile — more difficult than ever. N Eng J Med. 2008;359:1932-1940.

8 Linevsky J, Pothoulakis C, Keates S, et al. CP. IL-8 release and neutrophil activation by Clostridium difficile toxin-exposed monocytes. Am J Physiol. 1997; 273:G1333-G1340.

9 Warny M, Vaerman JP, Avesani V, and Delmée M. Human antibody response to Clostridium difficile toxin A in relation to clinical course of infection. Infect Immun.1994;62: 384-389.

10 Gravel D, Miller M, Simor A et al. Health care-associated Clostridium difficile infection in adults admitted to acute care hospitals in Canada: a Canadian Nosocomial Infection Surveillance Program Study. Clin Infect Dis. 2009;48:568-576.

11 Dubberke ER, Butler AM, Yokoe DS et al. Multicenter study of Clostridium difficile infection rates from 2000 to 2006. Infect Control Hosp Epidemiol. 2010;31:1030-1037.

12 Bauer MP, Notermans DW, van Benthem BHB, et al. Clostridium difficile infection in Europe: a hospital-based survey. The Lancet 2010;377(9759):63-73.

13 Beaugerie L, Flahault A, Barbut F, et al. Study Group.Antibiotic-associated diarrhoea and Clostridium difficile in the community. Aliment Pharmacol Ther.2003 Apr 1;17(7):905-912.

14 Wilcox MH, Mooney L, Bendall R, Settle CD, and Fawley WN. A case-control study of community-associated Clostridium difficile infection. J Antimicrob Chemo. 2008;62(2):388-396.

15 CDC, Vital Signs: Preventing Clostridium difficile Infections. MMWR March 9, 2012 61(09);157-162 available at http://www.cdc.gov/mmwr/preview/mmwrhtml/mm6109a3.htm

16 Crobach MJ, Dekkers OM, Wilcox MH, and Kuijper EJ. European Society of Clinical Microbiology and Infectious Diseases (ESCMID): data review and recommendations for diagnosing Clostridium difficile-infection (CDI) 2009. Clin Microbiol Infect. 15:1053–1066.

17 Cohen MB. Clostridium difficile infections: emerging epidemiology and new treatments. J Pediatr Gastroenterol Nutr. 2009;48 Suppl 2:S63-5.:S63-S65.

18 Karas JA, Enoch DA, Aliyu SH. A review of mortality due to Clostridium difficile infection. J Infect. 2010;61:1–8.

19 Drekonja D. Comparative Effectiveness of C. difficile treatments, Annals of Internal Medicine, 20 Dec 2011.

20 Kyne L, Warny M, Qamar A, and Kelly CP. Asymptomatic carriage of Clostridium difficile and serum levels of IgG antibody against toxin A. NEJM 2000;342:390-397.

21 Kyne L, Warny M, Qamar A, and Kelly CP. Association between antibody response to toxin A and protection against recurrent Clostridium difficile diarrhoea. The Lancet 2001;357:189-193.

22 Solomon K and Kyne L. Clostridium difficile-associate colitis: role of the immune response and approach to treatment.Colitis 2012..Fukata (ed.), ISBN 978-953-307-799-4, InTech, available from: http://www.intechopen.com/books/colitis/clostridium-difficile-associated-colitis-role-of-the-immune-response-and-approach-to-treatment

23 Aboudola S, Kotloff KL, Kyne L, Warny M, Kelly EC, Sougioultzis S, et al. Clostridium difficile vaccine and serum immunoglobulin G antibody response to toxin A. Infect Immun 2003;71(3) :1608-1610.

24 Kotloff KL, Wasserman SS, Losonsky GA, et al. Safety and immunogenicity of increasing doses of a Clostridium difficile toxoid vaccine administered to healthy adults. Infect Immun. 2001;69(2): 988 995.

25 Sougioultzis S, Kyne L, Drudy D, et al. Clostridium difficile toxoid vaccine in recurrent C. difficile-associated diarrhea. Gastroenterology. 2005 Mar;128(3):764-770.

26 Babcock GJ, Broering TJ, Hernandez HJ, et al. Human monoclonal antibodies directed against toxins A and B prevent Clostridium difficile-induced mortality in hamsters. Infect Immun. 2006;74(11) :6339-6347.

27 Leav BA, Blair B, Leney M, et al. Serum anti-toxin antibody correlates with protection from recurrent Clostridium difficile infection (CDI). Vaccine 2010;28(4):965-969.

28 Lowy I, Molrine DC, Leav BA, et al. Treatment with monoclonal antibodies against Clostridium difficile toxins. N Engl J Med. 2010 Jan 21;362(3):197-205.

29 Greenberg R, Marbury TC, Foglia G, Warny M. Phase I dose finding studies of an adjuvanted Clostridium difficile toxoid vaccine. Vaccine 2012; 30:2245-2249.

30 Foglia G, Shah S, Luxemburger C, Pietrobon PJF. Clostridium difficile: development of a novel candidate vaccine. Vaccine 2012.;30:4307-4309.

31 Plotkin S, Orenstein WA, Offit PA. Vaccines. 5th ed. Saunders (Elsevier); 2008:p 86.

32 Premier/i3/Safety surveillor databases analysis to support Phase III, Sanofi Pasteur, unpublished results

33 LabRx and Kaiser Permanente databases analysis to support Phase III, Sanofi Pasteur, unpublished results

34 Cohen SH, Gerding DN, Johnson S et al. Clinical practice guidelines for Clostridium difficile infection in adults: 2010 update by the society for healthcare epidemiology of America (SHEA) and the infectious diseases society of America (IDSA). Infect Control Hosp Epidemiol. 2010;31:431-455.

35 Eastwood K, Else P, Charlett A, and Wilcox M. Comparison of nine commercially available Clostridium difficile toxin detection assays, a real-time PCR assay for C. difficile tcdB, and a glutamate dehydrogenase detection assay to cytotoxin testing and cytotoxigenic culture methods. J Clin Microbiol. 2009 Oct; 47(10):3211-3217.

36 Jennison C and Turnbull. BW Group Sequential Methods with Applications to Clinical Trials. Chapman and Hall/CRC, 1999.

37 EQ-5D-3L Health Questionnaire, 1998 EuroQol Group.

38 Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. Scand J Gastroenterol. 1997;32 (9):920-924.

39 Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. Scand J Gastroenterol. 1997;32 (9):920-924.

40 Breslow NE, Holubkov R. Maximum likelihood estimation for logistic regression parameters under two-phase, outcome dependent sampling. Journal of the Royal Statistical Society Series B, Statistical methodology 1997;59:447-461.

41 Borgan O, Langholz B, Samuelsen SO, Goldstein L, Pogoda J. Exposure stratified case-cohort designs. Lifetime Data Anal 2000;6:39-58.