PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

(a)	Member State of notification	DE
(b)	Notification number	B/DE/16/PEI 2797
(c)	Date of acknowledgement of notification	21/06/2016
(d)	Title of the project	Safety and immunogenicity study of a *Clostridium difficile* vaccine in healthy adult volunteers
(e)	Proposed period of release	From 01/08/2016 until 30/10/2016

2. Notifier

Name of institution or company: Royal Holloway and Bedford New College, University of London

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid		(.)
RNA virus		(.)
DNA virus		(.)
bacterium		(x)
fungus		(.)
animal		
-	mammals	(.)
-	insect	(.)
-	fish	(.)
-	other animal	(.)

specify phylum, class …

(b) Identity of the GMO (genus and species)

Bacteria/Firmicutes/Bacilli/Bacillales/*Bacillaceae*/*Bacillus*/*Bacillus subtilis.* The CDVAX drug substance has the following nomenclature: CDVAX drug substance. It can also be referred to as CDVAX spores, PP108 spores or *B. subtilis* PP108 spores.

(c) Genetic stability – according to Annex IIIa, II, A(10)
Highly stable

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?
Yes (.) No (x)
If yes, insert the country code(s) …

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?
Yes (.) No (x)
If yes:
- Member State of notification …
- Notification number B/../../…

Please use the following country codes:
Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?
Yes (.) No (x)
If yes:
- Member State of notification …
- Notification number B/../../…

7. Summary of the potential environmental impact of the release of the GMOs.
An environmental impact is not expected as the release of the GMO (CDVAX) is limited to 12 healthy volunteers in the hospital settings. According to the environmental risk assessment there is negligible environmental risk associated with the CDVAX product.

B. Information relating to the recipient or parental organism from which the GMO is derived

1. Recipient or parental organism characterisation:

(a) Indicate whether the recipient or parental organism is a:

(select one only)

viroid (.)
RNA virus (.)
DNA virus (.)
bacterium (x)
fungus (.)
animal
- mammals (.)
- insect (.)

- fish (.)
- other animal (.)
  (specify phylum, class) …

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	…
(ii)	genus	*Bacillus*
(iii)	species	*subtilis*
(iv)	subspecies	Marburg
(v)	strain	PY79
(vi)	pathovar (biotype, ecotype, race, etc.)	Bacilli
(vii)	common name	*Bacillus subtilis*

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (.) No (x) Not known (.)

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (.)

If yes, indicate the type of ecosystem in which it is found:

Atlantic ..
Mediteranean ..
Boreal ..
Alpine ..
Continental ..
Macaronesian ..

(ii) No (x)
(iii) Not known (.)

(c) Is it frequently used in the country where the notification is made?
Yes (.) No (x)

(d) Is it frequently kept in the country where the notification is made?
Yes (.) No (x)

4. Natural habitat of the organism

(a) If the organism is a microorganism

water (.)
soil, free-living (.)
soil in association with plant-root systems (.)
in association with plant leaf/stem systems (.)

other, specify *Bacillus subtilis* can inhabit the gastrointestinal tract of humans, other mammalians, aquatic animals or insects.

(b) If the organism is an animal: natural habitat or usual agroecosystem:

…

5. (a) Detection techniques

PY79 can be detected by sequencing of its 16S rRNA or *gyrA* sequence (Hengstmann *et al*., 1999).

(b) Identification techniques

PY79 can be identified by sequencing of its 16S rRNA or *gyrA* sequence (Hengstmann *et al*., 1999).

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?

Yes (.) No (x)

If yes, specify

…

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (.) No (x) Not known (.)

If yes:

(a) to which of the following organisms:

humans (.)
animals (.)
plants (.)
other (.)

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC

PY79 is not known to cause any pathogenicity. It does not have pathogen vectors, and is not known to activate latent viruses. PY79 could inhabit the gastrointestinal tract of humans, other mammalians, aquatic animals or insects.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

PY79 is a laboratory strain that is no found in the environment. However, in natural ecosystems it is expected that PY79 would behave similarly to other *B. subtilis* strains. In favourable conditions, the normal life cycle of *B. subtilis* consists of asexual reproduction by binary fission, producing vegetative cells, the generation time being approximately 2 hours. In unfavourable conditions, *B.*

subtilis vegetative cells develop spores (also called endospores), which are considered as a means of perennation and dispersal, rather than reproduction. Sexual reproduction by transformation, conjugation or transduction is possible but rare.

(b) Generation time in the ecosystem where the release will take place:

In favourable conditions, the generation time of vegetative cells is approximately 2 hours.

(c) Way of reproduction: Sexual .. Asexual x

(c) Factors affecting reproduction:

If exposed to appropriate nutrients and environmental conditions, such as in an animal's gastrointestinal tract, PY79 can germinate and allow the outgrowth and resumption of vegetative cells (Hong *et al*., 2005). Vegetative *B. subtilis* cells will not survive outside of the host more than a few hours. PY79 spores are susceptible to high temperatures (typically over 100°C). In typical environmental conditions, PY79 vegetative cell integrity is strongly affected by sunlight, pH, temperature and absence of nutrients, and PY79 vegetative cells will not survive more than a few hours in the environment.

PY79 spores and vegetative cells are destroyed by 10% sodium hypochlorite (standard household bleach), autoclaving and incineration. It can also be destroyed by 5% sodium hydroxide solution, ionizing radiation, or by plasma-activated hydrogen peroxide. In addition, PY79 vegetative cells can be destroyed by ethanol or by UV light. Moreover, antimicrobial susceptibility testing was realized on a panel of standard antibiotics, and the *B. subtilis* PY79 strain is susceptible to various antibiotics, including gentamycin, neomycin, ampicillin, vancomycin and rifampicin (Hong *et al*., 2008).

9. Survivability

(a) ability to form structures enhancing survival or dormancy:

(i)	endospores	(x)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (funghi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify …	

(b) relevant factors affecting survivability:

If exposed to appropriate nutrients and environmental conditions, such as in an animal's gastrointestinal tract, PY79 can germinate and allow the outgrowth and resumption of vegetative cells (Hong *et al*., 2005). Vegetative *B. subtilis* cells will not survive outside of the host more than a few hours.

PY79 spores are susceptible to high temperatures (typically over 100°C). In typical environmental conditions, PY79 vegetative cell integrity is strongly affected by sunlight, pH, temperature and absence of nutrients, and PY79 vegetative cells will not survive more than a few hours in the environment.

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10. (a) Ways of dissemination
Faecal excretion from vaccinated subjects

(b) Factors affecting dissemination

PY79 spores are susceptible to high temperatures (typically over 100°C). In typical environmental conditions, PY79 vegetative cell integrity is strongly affected by sunlight, pH, temperature and absence of nutrients, and PY79 vegetative cells will not survive more than a few hours in the environment.

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)
…, B/../../…

C. Information relating to the genetic modification

1. Type of the genetic modification

(i)	insertion of genetic material	(x)
(ii)	deletion of genetic material	(.)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify …	

2. Intended outcome of the genetic modification
Expression of antigenic sequences to generate a prophylactic vaccine response.

3. (a) Has a vector been used in the process of modification?
Yes (x) No (.)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?
Yes (x) partially No (.)

If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector

plasmid (x)

bacteriophage		(.)
virus		(.)
cosmid		(.)
transposable element		(.)
other, specify	…	

(b) Identity of the vector
pPP052 plasmid vector: insertion vector containing the *cotB-tcdA*$_{26\text{-}39}$ construct (based on pDG1664 commercial vector).
pPP059 plasmid vector: insertion vector containing the *cotC-tcdA*$_{26\text{-}39}$ construct (based on pDG364 commercial vector).

(c) Host range of the vector
E. coli

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype
Yes (x) No (.)

antibiotic resistance	(x)
other, specify	Expression of the cotC-tcdA$_{26\text{-}39}$ and cotB-tcdA$_{26\text{-}39}$ inserts

Indication of which antibiotic resistance gene is inserted
Chloramphenicol, erythromycin and lincomycin resistance.

(e) Constituent fragments of the vector

The inserted chimeric sequences are individually and stably integrated into the genome of *B. subtilis* strain PY79 by a recombination event at the *amyE* and *thrC* sites. The inserted sequences include the antigen encoding cassette and either erythromycin and lincomycin or chloramphenicol resistance genes (*erm* and *cat*, respectively) for antibiotic selection of transformed cells. There are no known virulence features associated with these inserts. Both the plasmid origin of replication and the β-lactamase gene from each plasmid are lost during the recombination event, which results in a stable genomic integration event.

(f) Method for introducing the vector into the recipient organism

(i)	transformation	(x)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(.)
(vi)	other, specify	

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

(i)	transformation	(.)
(ii)	microinjection	(.)
(iii)	microencapsulation	(.)
(iv)	macroinjection	(.)

(v) other, specify …

6. Composition of the insert

(a) Composition of the insert
There are two insertion events: the cotB-tcdA$_{26\text{-}39}$ insert includes an rRNA adenine *N*-6-methyltransferase gene (*erm*) for erythromycin and lincomycin resistance (MLS^R) and the cotC-tcdA$_{26\text{-}39}$ insert includes a chloramphenicol acetyl transferase gene (*cat*) for chloramphenicol resistance (Cm^R), for antibiotic selection of transformed cells. Both antibiotic resistance markers have been chosen because of their limited use in the clinic or for animal welfare.

(b) Source of each constituent part of the insert
The non-toxic part of the *C*-terminal domain of toxin A (TcdA$_{26\text{-}39}$) of *Clostridium difficile* (*C. difficile*) strain 630 is the antigenic component of the product. This is fused to two PY79 *B. subtilis* spore coat proteins (CotB or CotC). The two antibotic resistance marker genes (erythromycin and lincomycin resistance (MLS^R) and the chloramphenicol acetyltransferase gene) originated from the pDG364 and pDG1664 commercial vectors respectively.

(c) Intended function of each constituent part of the insert in the GMO
The *cotC-tcdA$_{26\text{-}39}$* and *cotB-tcdA$_{26\text{-}39}$* inserts allow the expression of two recombinant proteins that allow the expression of the TcdA$_{26\text{-}39}$ antigen on the surface of the *B. subtilis* spore.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (x)
- other, specify …

(e) Does the insert contain parts whose product or function are not known?
Yes (.) No (x)
If yes, specify …

D. Information on the organism(s) from which the insert is derived

1. Indicate whether it is a:

viroid (.)
RNA virus (.)
DNA virus (.)
bacterium (x)
fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)
(specify phylum, class) …

other, specify …

2. Complete name

Bacillus subtilis PY79

(i)	order and/or higher taxon (for animals)	…
(ii)	genus	*Bacillus*
(iii)	species	*subtilis*
(iv)	subspecies	Marburg
(v)	strain	PY79
(vi)	pathovar (biotype, ecotype, race, etc.)	Bacilli
(vii)	common name	*Bacillus subtilis*

Clostridium difficile 630

(i)	order and/or higher taxon (for animals)	…
(ii)	family name for plants	*Clostridium*
(iii)	genus	*Clostridium*
(iv)	species	*difficile*
(v)	subspecies	…
(vi)	strain	630
(vii)	cultivar/breeding line	ribotype 012
(viii)	pathovar	*Clostridium*
(ix)	common name	*Clostridium difficile*

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (x) for *Clostridium difficile 630* only No (.) Not known (.)

If yes, specify the following:

(b) to which of the following organisms:

humans	(x)
animals	(x)
plants	(.)
other	..

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism

Yes (x) No (.) Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):
Clostridium difficile strain 630 is a fully virulent, highly transmissible, drug resistant strain of *C. difficile*. In the absence of competitors in the human GIT, following treatment with antibiotics, *C. difficile* multiplies and overgrows, causing a disturbance of the bacterial flora resulting in enteric disease. Germination of *C. difficile* spores following antibiotic treatment allows the bacteria to proliferate, with the concurrent production of two toxins (toxin A and toxin B) that leads to severe diarrhoea and acute colitis. It is the leading cause of antibiotic-associated diarrhoea and

pseudomembranous colitis (Knoop *et al*., 1993). It has been shown that *C. difficile* can also become pathogenic after treatment with proton pump inhibitors or histamine H2 antagonists. However, it is a portion of genomic DNA obtained from the donor, not the donor itself, which is used to generate the GMO.

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?

Yes (.) No (x)

If yes, specify Not applicable as it is a portion of genomic DNA obtained from the *C. difficile* donor, not the donor organism itself, which is used to generate the GMO.

5. Do the donor and recipient organism exchange genetic material naturally?

Yes (.) No (x) Not known (.)

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

(a) is the GMO different from the recipient as far as survivability is concerned?

Yes (.) No (x) Not known (.)

Specify …

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?

Yes (.) No (x) Unknown (.)

Specify …

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?

Yes (.) No (x) Not known (.)

Specify …

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?

Yes (.) No (x) Not known (.)

Specify …

2. Genetic stability of the genetically modified organism

Highly stable, due to insertion of genetic material into the bacteria's genome and loss of the plasmid origins of replication. The recominant sequence is therefore an integral part of the host DNA.

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?

Yes (.) No (x) Unknown (.)

(a) to which of the following organisms?

humans (.)
animals (.)
plants (.)
other …

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)

Like the parental organism, the GMO is not pathogenic. In a natural setting, the GMO can be present as spores, which is the dormant form, and as vegetative cells, that do not survive more than a few hours in the environment. In preclinical work supporting this study, the elimination study showed a low level of replication and dissemination. Due to the non-pathogenicity and limited replication of the GMO in the environment, the environmental impact is thought to be negligible.

(i) Toxic or allergenic effects of the GMOs and/or their metabolic products

The GMO was well tolerated in the preclinical studies. Two toxicology studies (GLP and non-GLP) were conducted in mice to show the safety of CDVAX. In both studies, CDVAX was well tolerated and showed no treatment-related adverse events. Like the parental organism PY79, the GMO is non-pathogenic.

(ii) Comparison of the modified organism to the donor, recipient or (where appropriate) parental organism regarding pathogenicity

PY79 is not pathogenic and PP108 is not expected to be, based on its similarities with PY79 and the non-pathogenicity of the inserted sequences.

(iii) Capacity for colonisation

Preclinical data indicate that the GMO can colonize the GIT of mice, however at a low level and during a limited time.

(iv) If the organism is pathogenic to human who are immunocompetent

The GMO has not been administered to humans yet. The parental strain PY79 is not known to be pathogenic to immunocompetent humans, and the GMO is not expected to be either. Preclinical data including pharmacology, toxicology and elimination studies in rodents indicated no adverse events related to the GMO. In addition, there have been over 100 clinical trials including healthy subjects or patients (adults and children) treated with pharmaceuticals or probiotics containing other natural or recombinant *B. subtilis* strains (see Environmental Risk Assessment, Edition n°1, Section 2.2) with an excellent safety profile and no adverse events related to *B. subtilis* reported. Moreover, several *B. subtilis* strains carry the GRAS and QPS status.

(v) Other product hazards

None known.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment

The detection of the GMO (PP108 spores) can be verified by culture selective plating using replica plating on LB agar, with or without chloramphenicol and erythromycin.

(b) Techniques used to identify the GMO

The identity of the GMO (PP108 spores) can be determined by culture selective plating as described above. The identity of the GMO (PP108 spores) can also be determined by the presence of $TcdA_{26-39}$ antigens using western blotting. This test was validated and is used as a release test for the IMP.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)

The purpose of the release is for a first-in-man clinical study in twelve (12) healthy male volunteers. If the vaccine is successful it will be a very important public health tool.

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?

Yes (.) No (x)

If yes, specify …

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
Study site: Nuvisan GmbH, Wegenerstraße 13, 89231 Neu-Ulm, Germany

(b) Size of the site (m^2): n/a m^2
(i) actual release site (m^2): n/a m^2
(ii) wider release site (m^2): n/a m^2

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
…

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
…

4. Method and amount of release

(a) Quantities of GMOs to be released:

No more than 1×10^{12} CFU.

(b) Duration of the operation:

The start of the study is planned for 3Q-2016. Enrolment is planned for 6 to 8 weeks following the start of the study. Administration consists of one oral administration of CDVAX every two weeks during 6 weeks (*i.e.* on day 0, 14, 28 and 42), for a total of 4 administrations per subject.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release

Preparation of the treatment will take place in a standard hospital room and be realized by authorized medical staff. Onsite preparation of the treatment consists of extraction of 1 ml of the CDVAX drug product from 5 tubes (5 tubes contained in one box of the CDVAX drug product) with a syringe with needle, for a total volume of 5 ml. The needle is then removed from the syringe. The use of a single syringe with needle to extract the IMP from the tube was selected to allow minimum liquid remaining in the tube. Administration of the IMP will be realized by authorized medical staff in a conventional hospital room according to Good Clinical Practices and the study protocol. Administration consists of pouring the 5 ml of IMP contained in the syringe (with the needle removed) directly into the mouth of the subject. The primary mode of containment during the preparation and administration procedures is application of standard precautions for biohazard materials. In the hospital room, the medical staff performing the procedures will wear gloves and gown or lab coat. The preparation and administration by design, and best practice, minimize the opportunity for liquid waste, aerosol generation and needle sticks.

The investigational site abides by all European Union, country and self-imposed guidelines regarding the conduct of clinical trials, as well as the appropriate biosafety regulations required by the European Medicinal Agency for vaccine medicinal research. The sponsor believes that research conducted within this framework adequately mitigates the risks of such research to the public health and therefore no additional measures will be undertaken.

Destruction of unused IMP and destruction or decontamination of all materials that may have been contaminated by the IMP is discussed in Section V.C "Waste treatment" of Annex IIIa.

5. Short description of average environmental conditions (weather, temperature, etc.)
The GMO will be administered in a hospital room and appropriate safety measures to will be maintained to prevent spread.

6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release.
The GMO has not been administered to humans yet. However many *Bacillus subtilis* strains including those contain multiple antibiotic resistance markers are routinely used in the human and animal products including foods and health suppliments. These include Primal Defense™, Bactisporin™ and Bio-Kult®.

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Name of target organism (if applicable)

(i)	order and/or higher taxon (for animals)	Primate
(ii)	family name	Hominidae
(iii)	genus	Homo

(iv)	species	sapiens
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	…

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

The anticipated mechanism is expected to be similar to the mechanism demonstrated in the preclinical programme. CDVAX consists of *B. subtilis* spores that express two recombinant fusion proteins on their surface. The two recombinant proteins comprise $TcdA_{26\text{-}39}$ from *C. difficile* fused to one of two *B. subtilis* spore coat proteins (CotC or CotB) enabling the display of the toxin A recombinant protein $TcdA_{26\text{-}39}$ on the outer surface of the spores. CDVAX will be delivered orally as a liquid suspension to prevent primary CDI. This direct delivery of the vaccine to the mucosal surface of the GIT will ensure the induction of a mucosal immune response (Neutra *et al*., 2006). The ease of oral administration will ensure greater patient compliance and is particularly attractive in a hospital setting.

Non-clinical proof of concept has been achieved through confirmation of an immune response induced by oral administration of CDVAX in rodents that is sufficient to protect against *C. difficile* challenge. Both oral and sublingual administration routes show a strong induction of anti-toxin A mucosal immunity (IgA). Oral/sublingual vaccination (four or five administrations) with CDVAX induced significant systemic and mucosal immunity, with the appearance of both mucosal (IgA) and systemic (IgG) antibodies. These antibodies were subsequently shown to have the capacity to neutralise *C. difficile's* toxin A cellular toxicity. The immune response induced by vaccination with CDVAX is sufficient to protect rodents against *C. difficile* challenge.

The end result of the release of the GMO is to vaccinate the target organisms against *C. difficile* infection. As shown by preclinical data, the result of interaction of CDVAX with the target organisms is expected to effectively induce a mucosal immunity and generate neutralising antibodies against toxin A able to prevent *C. difficile* primary infection and recurrence.

3. Any other potentially significant interactions with other organisms in the environment
n/a

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (x) Not known (.)
Give details
…

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
n/a

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

(i)	order and/or higher taxon (for animals)	…
(ii)	family name for plants	…
(iii)	genus	…
(iv)	species	…
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	…

7. Likelihood of genetic exchange in vivo

   (a) from the GMO to other organisms in the release ecosystem:
   Negligible

   (b) from other organisms to the GMO:
   Negligible

   (c) likely consequences of gene transfer:
   The two expression constructs provide no growth advantage. There are two antibiotic drug resistance marker genes. As many probiotic products that are already on the market contain multiple antibiotic drug resistance marker genes then the presence of two antibiotic drug resistance marker in CDVAX is not considered to provide any risk or advantage.

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):
   n/a

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
   n/a

H. Information relating to monitoring

1. Methods for monitoring the GMOs

In the phase I clinical study, the sponsor will monitor CDVAX shedding in faeces of the subjects 1 month after the last administration (day 70), and 6 months later. CDVAX, as well as potential PP108 vegetative cells, will be detected in faeces by a culture selective plating method using agar plates with selection for chloramphenicol, erythromycin and lincomycin. This assay was used in the preclinical programme and showed that the elimination of the GMO from mice was almost complete in the first three days after oral administration, with at least a 4-log reduction observed between 4 hours and D3 after administration.

2. Methods for monitoring ecosystem effects
   n/a

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms

The donated genetic material to other organisms can be identified by PCR (polymerase chain reaction), using specific primers for *tcdA*$_{26\text{-}39}$, *cotB* and *cotC* (as described in the supplementary data of Permpoonpattana *et al*., 2011). Another method to identify the donated genetic material can be the sequencing of the inserted gene sequences, *cotB-tcdA*$_{26\text{-}39}$*-erm* and *cotC-tcdA*$_{26\text{-}39}$*-cat.* This method was validated as a characterization test in the specifications of the MCB and the drug product of CDVAX. These two methods could be applied to monitor the transfer of the donated genetic material to other organisms. However there is no plan to do so as transfer and impact to other organisms are negligible.

4. Size of the monitoring area (m^2)
n/a m^2

5. Duration of the monitoring

The health of subjects enrolled in the study will be monitored for six months over the course of the study.

While some PP108 spore shedding (or possible PP108 vegetative cell shedding) by subjects following administration is expected, there is negligible risk from shedding and exposure of family members or other casual contacts from PP108 spores or vegetative cells. Therefore shedding and effects will be monitored 1 month and 6 months after the last administration in the clinical study, and effects will also be monitored two weeks after each administration of CDVAX. There is also negligible risk of transfer of donated genetic material from the subject to other people or to other organisms, therefore the transfer of donated genetic material will not be investigated.

6. Frequency of the monitoring

On days 0, 14, 28 and 42 of the study, subjects will undergo an oral administration of the IMP. On these days and 24 hours later, as well as two weeks after the last administration, the subjects will be submitted to a battery of safety and immunogenicity assessments, followed by two follow-up visits (1 month after the last administration and 6 months later). During the follow-up period, information on clinical events will be collected (pharmacovigilance procedure). All subjects will be observed and followed for a minimum of 8 cumulative months.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

All disposable materials (including but not limited to gloves, tubes, syringes and needles) that come into contact with the GMO will be disposed of as hazardous biological materials according to individual institutional practices and policies. In general the materials will be disposed of in sharps containers or biohazard bags and decontaminated by autoclave or incineration, or both.

The unused IMP, tube and screw cap will be disposed of as biohazardous waste and handled the same way as the disposable materials that come into contact with the GMO.

Non-disposable materials, equipment and surfaces will be decontaminated with a 10% sodium hypochlorite (standard household bleach). Some non-disposable materials may be autoclaved.

2. Post-release treatment of the GMOs
…

3. (a) Type and amount of waste generated

Generated waste corresponds to disposable materials including gloves, syringes and needles, and to unused IMP, tube and cap. The amount of waste is typical of an oral administration procedure where the waste is considered as biohazardous material.

For the investigational site, the amount of liquid waste is expected to be close to 0 ml of IMP, as the complete volume of each tube is expected to be administered to the subjects. The use of a syringe with needle to extract the IMP from the tube was selected to allow minimum liquid remaining in the tube. During administration, one syringe and one needle will come into contact with the IMP. The remaining waste generated is consistent with a typical oral administration procedure resulting in the generation of a few biohazardous waste (disposable gloves, used boxes, tubes and caps of the IMP).

3. (b) Treatment of waste

It is envisaged to treat with 10% sodium hypochlorite (standard household bleach) for 10 minutes all surfaces and non-disposable instruments (that cannot be autoclaved) which were potentially contaminated with the IMP. All disposable materials that come into contact with the IMP will be disposed of as hazardous biological materials according to individual institutional practices and policies. In general the disposable materials will be disposed in sharp containers or biohazard bags and decontaminated by autoclave or incineration, or both. Unused IMP will be destroyed by, for example treatment with bleach, autoclaving and/or incineration, but in accordance with the institution's applicable practices and policies.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread

Personal protective equipment including gloves and gown or lab coat should be worn when working with the GMO. If skin or eyes are exposed they should be rinsed with copious amounts of water. In the case of a needle stick or ingestion, a physician should be consulted. An antibiotic treatment can be prescribed to eradicate the GMO (*e.g.* gentamycin, tetracycline, neomycin, ampicillin, vancomycin, ciprofloxacin and/or rifampicin).

2. Methods for removal of the GMO(s) of the areas potentially affected

If the IMP is spilled or otherwise dispersed during the preparation or administration, cleaning procedures should be performed in accordance with standard practices for cleaning up biohazard waste spills. Accidental spills will be cleaned up according to standard local practice.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
Not required

4. Plans for protecting human health and the environment in the event of an undesirable effect
The health of the volunteers in the trial will be actively and closely monitored for the duration of the study. Any symptoms will be clinically managed by the study physicians as appropriate.