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 GB
(b) Notification number B/GB/17/R50/01
(c) Date of acknowledgement of notification 25/07/2017
(d) Title of the project
Experimental challenge of the human nasopharynx with recombinant *Neisseria lactamica* expressing the meningococcal type V autotransporter protein, *Neisseria* Adhesin A (NadA).

(e) Proposed period of release From 01/11/2017 until 30/11/2018

2. Notifier

Name of institution or company:
The University of Southampton
University Road
Southampton SO17 1BJ
United Kingdom

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 Proteobacteria, betaproteobacteria

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

There are two GMOs in use in this study. The first is a NadA-expressing *Neisseria lactamica* strain, a derivative of the wild type strain Y92-1009. The GMO has been constructed so that a gene expression cassette, containing an Nlac codon-optimised version of the *nadA* gene (NMB_1994) and a copy of the endogenous *lacZ* gene, coding for β-D-galactosidase, is stably integrated into the bacterial chromosome at an intergenic locus (*Neisseria* Heterologous Construct Insertion Site number 1, NHCIS1) (hereafter, strain 4NB1). The second GMO is the control strain, also derived from wild type strain Y92-1009, which has been modified at the same site and using the same protocol as the NadA-expressing strain, but contains a gene expression cassette with only the endogenous *lacZ* gene (hereafter, strain 4YB2).

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

In reference to Annex IIIa, section II. A. a) 10, a major feature of the genomes of the *Neisseriaceae* is the abundance of repetitive sequences, including but not limited to *Neisseria* DNA uptake sequences, Correia Repeat Enclosed Elements (CREE), dRS3 elements and homo- and hetero-polymeric nucleotide tracts. In addition to serving functional roles, these sequences are responsible for frequent chromosomal rearrangements in these bacteria, which are evident at all taxonomic levels (i.e. between species, between strains of the same species and between isolates of the same distinct lineages). Consistent with this, we have observed a low number of chromosomal rearrangements in the GMO strains, relative to a complete, closed reference genome derived from DNA extracted from wild type *N. lactamica* strain Y92-1009 (analysis performed using progressive Mauve). The sizes of the rearrangements vary considerably. In both GMOs the NHCIS1 locus, where the gene expression construct is located, lies within a Local Colinear Block (LCB) of the reordered assembly and is unaffected by chromosomal breakpoints, which could otherwise potentially modify the regulatory sequences controlling expression of the *nadA* and/or *lacZ* genes. That these repeat-driven rearrangements naturally occur within the Neisseriaceae suggests that currently circulating strains of *N. lactamica* also demonstrate chromosomal mosaicism relative to one another. Given that *N. lactamica* is non-pathogenic, it is therefore very unlikely that these rearrangements in the GMO will result in increased pathogenicity and are not a cause for concern. The analysis also showed that, despite the rearrangement of gene order, the GMOs contain the same gene content as the parental antecedent. Following repeated sub-culture of the GMO strains over 28 days *in vitro*, a similar number of rearrangements were evident; however the gene content was unaffected and the GMOs continued to contain β-galactosidase Specific Activity (strains 4NB1 and 4YB2) and to express NadA to similar levels (strain 4NB1).

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.

Neisseria lactamica is a human adapted nasopharyngeal commensal that does not survive outside of its sole biological niche. The environmental impact of the release of the GMOs, as pertains to the impact of the organism on the air, land, sea, flora and fauna of the United Kingdom is therefore negligible.

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) Proteobacterium, betaproteobacterium

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	Neisseriaceae
(ii)	genus	*Neisseria*
(iii)	species	*Neisseria lactamica*
(iv)	subspecies	…
(v)	strain	Y92-1009
(vi)	pathovar (biotype, ecotype, race, etc.)	N/A
(vii)	common name	*Neisseria lactamica* (Nlac)

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:

Yes (X) No (.) Not known (.)

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

(i) Yes (X)

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

NB: Exclusively human nasopharyngeal commensal, endemic globally:

Atlantic	X
Mediteranean	X
Boreal	X
Alpine	X
Continental	X
Macaronesian	X

(ii) No (.)

(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 (X) No (.)

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	Commensal bacterium exclusive to human nasopharynx.

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

N/A

5. (a) Detection techniques

Following intranasal inoculation of volunteers, at the appropriate time points throat swabs and nasal washes will be performed. These samples will be used to try and culture viable *Neisseria* species on both GC and chocolate agar plates, on which routine laboratory culture of this species is performed.

(c) Identification techniques

Putative *Neisseria* colonies (identified by colony morphology) will be identified as *Neisseria lactamica* in the first instance by metabolism of exogenously applied 5-bromo-4-Chloro-3-Indolyl β-D-Galactopyranoside (X-gal) to form a blue breakdown product. Verification will be made either with mass spectrometry (MALDI-TOF), for which there is a strong fragmentation fingerprint, or using a panel of conventional biochemical tests (API NH, bioMerieux). Species level identification of *N. lactamica* is possible using this panel of tests (a '5041' result relates specifically to Nlac).

Neisseria lactamica colonies thus identified will be specifically identified as the genetically-modified strains by PCR of the NHCIS1 locus. PCR amplification of the NHCIS1 locus from the GMOs will produce products of either 6.7 kb (4NB1) or 5.4 kb,(4YB2) whereas amplification of the same locus from wild type Nlac isolates will produce a 2.2 kb product. The combination of these identification techniques provides for a robust means of identifying the GMOs from a complex, polymicrobial sample.

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

Yes (.) No (X)

The UK ACDP has not categorised *N. lactamica*, therefore wild type *N. lactamica* should be considered a *group one biological agent* under the European Economic Community (EEC) classification for the protection of workers with biological agents [Directive 2000/54/EC]. Work with GM-*N. lactamica* has been risk assessed by the University of Southampton Genetic Modification & Biosafety Committee (GMBSC) and, following notification of the UK Health and Safety Executive is routinely handled as activity *class 2*.

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

N/A

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

No data exists on the generation time of *N. lactamica* in the human nasopharynx, but various investigations on a variety of growth media suggest a generation time longer than that of *E. coli*. The generation time of *N. lactamica* in TSB liquid medium supplemented with 0.2 % yeast extract at 37 ^{o}C was calculated to be *74 minutes* in our hands. The generation time *in vivo* is therefore expected to be longer than 74 minutes.

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

The release will take place directly into the human nasopharnx of study volunteers, therefore see 8a.

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

(d) Factors affecting reproduction:

Nutrient availability, presence of competitors/composition of nasopharyngeal microbiome, antibiotic usage by study participant.

9. Survivability

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

(i)	endospores	(.)
(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:

N. lactamica is a fastidious, human-adapted organism that is terminally adapted to life in its sole biological reservoir. The introduction of an outer membrane adhesin (strain 4NB1) has, at most, the potential to modify the organism's interaction with its preferred niche and will not confer a survival advantage to the GMO over other strains of *N. lactamica* in other environments. Factors such as immunological status of volunteers and composition of the nasopharyngeal microbiome are factors that will impact survivability.

10. (a) Ways of dissemination

The GMO is `non-attenuated` (but the wild type is a commensal and non-virulent) and is expected to be able to survive within its biological niche (the human nasopharynx) following colonisation. *N. lactamica* is spread by person to person transmission through exchange of respiratory and throat secretions (saliva) or during close and/or lengthy contact. There is no evidence for airborne transmission of the bacterium through inhalation of respiratory droplets. Following release, there is a chance that close contacts of study participants (i.e. sleeping partners) will also become carriers of the GMO.

(b) Factors affecting dissemination

The GMO is an exclusively human commensal bacterium with no other known reservoir. *N. lactamica* has extremely limited survivability outside of its biological niche. Although there is currently no supportive data, it is assumed that the transmission of the GMO will be similar to *N. meningitidis* – about which there is considerable information on transmission. Transmission of *N. meningitidis* between humans occurs with the greatest frequency in household contacts (especially bedroom-sharers), sexual contacts, and in people who attend pubs and clubs, or live in University dormitories/halls of residence. The number of close interpersonal contacts made by an inoculated volunteer will therefore influence the likelihood of GMO dissemination, with increasing numbers of close contacts correlating with an increased chance of spread. To limit transmission, we will train the participants in infection control and request that they do not attend pubs and clubs in the 2 weeks after discharge (because there is evidence that onward transmission between humans occurs mostly in the first 2 weeks after acquisition) and monitor (and offer eradication treatment) to bedroom contacts. Additionally, the incidence of meningococcal disease peaks during the winter, suggesting an increased rate of *N. meningitidis* transmission during the winter months. However, no data has been produced to date on whether this can be extrapolated to transmission of *N. lactamica.*

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

The purpose of the genetic modification is to construct a strain of the exclusively human, nasopharyngeal commensal bacterium, *Neisseria lactamica* (Nlac) that expresses on its surface the outer membrane protein, *Neisseria* Adhesin A (NadA). NadA is an adhesin protein found in the close relative of Nlac, *Neisseria meningitidis* (Nmen), which is the causative agent of meningococcal disease. The genetically modified organism (GMO) will be used to investigate the role of NadA in the colonisation of the nasopharynx and associated immune responses in a controlled human bacterial challenge. We anticipate the expression of the NadA protein to provide an advantage for colonisation to Nlac, allowing the bacterium to utilise the as-yet unidentified receptor for NadA on the surface of human epithelial cells for binding. Colonisation of the human nasopharynx by Nlac has been shown by the applicants to displace circulating strains of Nmen, reducing the levels of Nmen carriage by an amount comparable to polysaccharide-conjugate vaccines. Increasing the efficiency of Nlac colonisation could plausibly impact on the frequency of carriage of Nmen, with a concomitant reduction in the incidence of meningococcal disease. The primary objective of the human challenge experiment is to determine whether intranasal administration of the GMO is truly safe (as expected), whilst secondary objectives are determining the impact of NadA expression on the frequency of nasopharyngeal colonisation by GM-Nlac and the type(s) of immune responses generated locally and systemically to these bacteria.

To provide a suitable control for this study, another genetically modified strain was derived in which the screening marker, the *lacZ* gene, was placed into the same locus as the *nadA* and *lacZ* genes in the NadA-expressing strain.

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

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?
Yes (.) 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 (.)
bacteriophage (.)
virus (.)
cosmid (.)
transposable element (.)
other, specify …

(b) Identity of the vector
…

(c) Host range of the vector
…

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

antibiotic resistance (.)
other, specify …

Indication of which antibiotic resistance gene is inserted
…

(e) Constituent fragments of the vector
…

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

(i) transformation (.)
(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 (X)
(ii) microinjection (.)
(iii) microencapsulation (.)
(iv) macroinjection (.)
(v) other, specify …

6. Composition of the insert

(a) Composition of the insert

All sequences in the constructs are known. There are two transformative constructs ('inserts'): NHCIS1::HAEC4:(Z)-*lacZ* (present in strain 4YB2) and NHCIS1::HAEC4:*nadA*-*lacZ* (present in

strain 4NB1). There are two genes present in the NHCIS1::HAEC4:*nadA*-*lacZ* cassette: *nadA*, coding for the *Neisseria* Adhesin A (NadA) protein, a type V autotransporter from Nmen strain MC58 (NMB_1994), and *lacZ*, coding for β-galactosidase. Only the *lacZ* gene is present in the NHCIS1::HAEC4:(Z)-*lacZ* cassette.

The construct in its entirety (6, 703 bp) consists of:

1. Sequence homologous to the NHCIS1 locus of *N. lactamica* strain Y92-1009 (5'ENDNHCIS1 and 3'ENDNHCIS1). This locus is defined as the 'intergenic' region between NLY_27080 and NLY_27100. In the construct, the locus has been bifurcated and flanks the gene expression cassette.

2. Two *Neisseria* DNA Uptake Sequences (DUS: 5' – GCCGTCTGAA – 3'), included to increase the frequency with which the construct is bound by the Type IV pilus of Nlac and internalised by the bacteria as part of natural competence.

3. A 200 bp upstream activator sequence (UAS) found immediately upstream of the *porA* gene in wild type *N. meningitidis* strains. This length of sequence has been shown to optimally enhance the activity of promoters when conjugated directly upstream of the -35 RNA Polymerase binding site.

4. A synthetic, hybrid promoter consisting primarily of sequence derived from the *porA* gene of wild type *N. meningitidis*, but wherein the homopolymeric guanosine nucleotide tract, a feature responsible for the regulation of expression of this gene by phase variation, has been replaced by sequence derived from the non-phase variable *porB* gene of *N. lactamica*.

5. A synthetic version of the *nadA* gene (NMB_1994), codon-optimised for expression in *N. lactamica* (CAI: 0.702) but still coding for the same amino acid sequence as the wild type gene present in *N. meningitidis* strain MC58. NB: In the NHCIS1::HAEC4:(Z)-*lacZ* Cassette, this gene is replaced with a 14 bp, non-coding linking sequence (5' – ATCTATTATATAAC – 3').

6. The promoter for the *N. lactamica* gene: α-2, 3 sialyltransferase (*lst*)

7. A copy of the endogenous *lacZ* gene from wild type *N. lactamica* (*lacZ*), coding for the cytoplasmic enzyme β-galactosidase.

8. A transcriptional terminator derived from the sequence immediately downstream of the *porB* gene from wild type *N. meningitidis* strain MC58.

(b) Source of each constituent part of the insert

The regions homologous to NHCIS1, the *lacZ* gene, the *lst* promoter and the *porB* terminator sequences were originally amplified from genomic DNA extracted from wild type *N. lactamica* strain Y92-1009. The synthetic hybrid promoter, the Upstream Activating Sequence, and the Nlac codon-optimised version of the *nadA* gene were designed *in silico* and synthesized as gene fragments (gBLOCKs, Integrated DNA Technologies). The DUS were incorporated into the construct by PCR utilizing extended primers.

(c) Intended function of each constituent part of the insert in the GMO

The regions homologous to NHCIS1 are intended to target the construct to the correct, intergenic chromosomal locus.
The DUS are intended to increase the frequency with which *N. lactamica* binds to and takes up the construct using their Type IV pili.
The UAS serves to enhance transcription from the synthetic hybrid promoter and increase the overall levels of NadA expression in the appropriate GMO.
The synthetic hybrid *porA/porB* promoter is intended to constitutively drive expression of the *nadA* gene to a high level.
The *nadA* coding sequence allows the GMO to express the NadA protein on its outer surface.
The *lst* promoter is intended to drive the expression of the *lacZ* gene, which is the screening marker used to identify putative transformants.
The *porB* terminator is intended to prevent the expression of downstream sequences during translation.

(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

(i) order and/or higher taxon (for animals) Neisseriaceae
(ii) family name for plants …
(iii) genus *Neisseria*
(iv) species *Neisseria meningitidis*
(v) subspecies …
(vi) strain MC58

(vii) cultivar/breeding line …
(viii) pathovar …
(ix) common name *Neisseria meningitidis* (Nmen)

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (X) No (.) Not known (.)
If yes, specify the following:

(b) to which of the following organisms:

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

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
Yes (.) No (X) Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):
…

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 (X) No (.)
If yes, specify
Neisseria meningitidis is categorised as a hazard group 2 human pathogen by the UK ACDP and in Annex III of Directive 90/679/EEC. *Neisseria meningitidis* must be handled at Containment level 2.

5. Do the donor and recipient organism exchange genetic material naturally?
Yes (X) No (.) 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 (.) Not known (X)
Specify
There is a paucity of information on the survivability of *Neisseria lactamica* outside of its biological reservoir. Environmental survival of other species of *Neisseria* has been investigated, with the most work having been performed on the 'pathogenic' *Neisseria*. However, the NadA gene product is an adhesin and is therefore not anticipated to confer a survival advantage outside of the human nasopharynx. We therefore expect this parameter to be unchanged. In terms of survival *within* the human nasopharynx, we predict that expression of NadA will confer a colonization advantage to the GMO, relative to the wild type or GM-control strains (i.e. we predict that a greater number of 4NB1 bacteria will be able to adhere to a greater number of epithelial cells *in vivo*, increasing the

likelihood of persistence within the nasopharynx at the epithelial surface). However, due to the fact that the NadA protein will be constitutively expressed and that we anticipate inoculated volunteers to serocovert against the NadA protein (it is, after all, an immunogenic component of the Bexsero vaccine), we predict that NadA expression may confer a survival disadvantage over the longer term. To summarise, we predict that an identical number of 4NB1 cells would adhere in greater numbers across more epithelial cells than strain 4YB2, but that the period of colonization could be shorter. In terms of displacing the meningococcus from nasopharyngeal carriage, as observed in previous human challenge experiments with wild type *N. lactamica,* this smaller window might prove sufficient; but there is currently no supportive data.

(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

The *in vitro* growth of the GMO strains in rich culture medium (TSB plus 0.2% yeast extract) is unaffected relative to wild type. In semi-defined growth media however (modified Frantz medium), the growth characteristics of strain 4NB1 differ from both 4YB2 and wild type *N. lactamica*, insofar as the GMO does not autoaggregate like the parental strain. The growth of 4YB2 and the wild type under these conditions are similar.

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

Yes (.) No (.) Not known (X)

Specify

As detailed in Section E.1.a, we predict a short term colonization advantage for strain 4NB1 relative to 4YB2, but a shorter period of colonization overall. NadA expression may result in a stronger interaction of the bacteria with epithelial cells; with less bacterial cells being shed into the saliva and other upper respiratory secretions. This would mean dissemination of the GMO among close contacts is less likely. Conversely, during the initial period of colonization, the increased abundance of NadA-expressing bacteria at the mucosal surface may result in a relative increase in the number of bacteria in saliva and other upper respiratory tract secretions, with the effect of enhancing the transmission of the NadA-expressing GMO to close contacts.

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

Yes (.) No (X) Not known (.)

Specify

The GMOs shows no evidence of increased pathogenicity compared to wild type in a murine model of intraperitoneal (i/p) infection, which is to say that the GMOs showed no evidence of pathogenicity. Wild type *N. lactamica* strain Y92-1009, and strains 4NB1 and 4YB2, were injected i/p at either a low (1.7×10^5 CFU) or a high (approx. 2.5×10^7 CFU) dose into NIH/OLA mice (10 per group) along with a bolus of holo-human transferrin as an exogenous iron source. An additional transferrin dose was injected after 24 h. Mice were monitored over the course of five days and all remained healthy. This is in contrast to i/p challenge with serogroup B *N. meningitidis* where 1/5 mice survived following a challenge with 2×10^6 CFU. There were no survivors following a dose of 2×10^7 CFU.

2. Genetic stability of the genetically modified organism

As detailed in Section A.3.c, the GMOs demonstrate repeat-mediated chromosomal rearrangements characteristic of the Neisseriaceae, but retain the same gene content as the wild type strain, Y92-1009. Over the course of 28 days *in vitro* passage of the GMOs, the expression of the exogenously added genes is maintained and the expression levels of the NadA adhesin remain consistent.

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)

…

4. Description of identification and detection methods

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

Following intranasal inoculation of volunteers, at the appropriate time points throat swabs and nasal washes will be performed. Throat swabs and nasal washes will be taken at Day 0 (challenge), daily during the 5 day admission, and then at 7, 10, 14, 28 and 90 days post-challenge. In addition, samples will be collected at any point the volunteers experience any adverse symptoms resulting in the necessity to terminate the study and treat the volunteer with eradicating antibiotics. It is expected that cultivation of the material from throat swabs will yield viable GMOs either 4 days or 7 days after inoculation, as this is what is most commonly observed after inoculation of the wild type organism.

(b) Techniques used to identify the GMO

Putative *Neisseria* colonies (identified by colony morphology) will be identified as *Neisseria lactamica* in the first instance by metabolism of exogenously applied X-gal to produce a blue breakdown product. Verification will be made either with mass spectrometry (MALDI-TOF), for which there is a strong fragmentation fingerprint, or using a panel of conventional biochemical tests (API NH, bioMerieux). Species level identification of *N. lactamica* is possible using this panel of tests (a '5041' result relates specifically to Nlac). *Neisseria lactamica* colonies thus identified will be specifically identified as the genetically-modified strains by PCR of the NHCIS1 locus. PCR amplification of the NHCIS1 locus from the GMOs will produce products of either 6.7 kb (4NB1) or 5.4 kb,(4YB2) whereas amplification of the same locus from wild type Nlac isolates will produce a 2.2 kb product. The combination of these identification techniques provides for a robust means of identifying the GMOs from a complex, polymicrobial sample.

F. Information relating to the release

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

The co-primary endpoints of the study will be (i) to establish the safety of nasal inoculation of healthy volunteers with a genetically modified strain of *Neisseria lactamica* expressing NadA, and (ii) to assess the NadA specific immunity in healthy volunteers following nasal inoculation with *Neisseria lactamica* expressing NadA (strain 4NB1). If applicable, the study may also suggest whether strain 4NB1 has an increased ability to colonise the nasopharynx. Assuming these endpoints are met, the future use of the GMO will be (1) to enable investigation of mucosal immunity to a meningococcal antigen during nasal carriage under controlled conditions (2) to test vaccines containing NadA for their ability to protect against colonisation by strains expressing the immunologically cognate antigen, (3) to use strain 4NB1 as the background strain for future bacterial medicines that can introduce a therapeutically beneficial gene into the human nasopharynx (e.g. for microbiome modification). A significant potential benefit accompanying the release is likely to be the elimination of *N. meningitidis* from nasopharyngeal carriage in Nlac-colonised volunteers. Because carriage of Nmen is pre-requisite for the development of meningococcal disease, removal of Nmen from carriage will preclude both the development of meningococcal disease in the colonised volunteer, and also the onward transmission of *N. meningitidis* to other, potentially vulnerable individuals.

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):

The release of the GMOs will take place in the NIHR-Wellcome Trust Clinical Research Facility (NIHR-WTCRF) in Southampton General Hospital, Southampton, UK (grid reference: SU397149). However, although we anticipate the majority of volunteers to be resident in the Southampton area for the duration of the study, there are no restrictions on their travel between visits. As such, transmission of the GMO beyond Southampton is possible.

(b) Size of the site (m^2): … m^2

(i) actual release site (m^2): 15 m^2

(ii) wider release site (m^2): UNKNOWN -

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

Not applicable.

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

Not applicable.

4. Method and amount of release

(a) Quantities of GMOs to be released:

Each volunteer will receive a total of 1ml of PBS, delivered as two 500 µl boluses (one into each nostril) containing a total of 100,000 (10^5) CFU of the appropriate GMO. Given we intend to

inoculate a maximum of 44 volunteers, the maximum number of each GMO to be released from the site is 2.2 x 10^6 CFU. However, we expect a subset of volunteers in each arm of the study to become colonized with the GMO and for the GMO, which is not attenuated, to replicate *in vivo*.

(b) Duration of the operation:

The study is expected to commence on the 1st of November 2017 (pending all necessary approvals) and will run for approximately 13 months (with an expected completion date of 30th November 2018).

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

Transmission beyond the household setting will be possible. We know that the wild type strain can persist in the nasopharynx for 6 months, and although onward transmission of *N.meningitidis* is most likely in the first 2 weeks after acquisition, transmission of the *N.lactamica* GMO at any time cannot be absolutely prevented. Therefore the action we propose is to limit as much as is reasonable, onward transmission into the community.

To that end, the challenge procedure will be carried out in one of the containment level 2 environmental chambers within the NIHR-WTCRF to assure the inoculum will be administered to the volunteer only, without posing any risk of infection to other people or the environment. The GMO will be delivered directly into the nares of volunteers by pipette. Staff will wear appropriate personal protective equipment during the challenge procedure. Before and after the challenge they will wash their hands and use an alcohol gel to clean their hands. After the challenge the room will be cleaned following NHS guidelines. All procedures to investigate the carriage of the GMO by the participant (throat swabs, nasal washes, nasal swabs, blood tests) will be undertaken in the containment level 2 environmental chambers within the NIHR-WTCRF.

In this study, all volunteers will be admitted to the NIHR-WTCRF for the first 5 days following inoculation and will be subject to 24 h clinical observation. Whilst volunteers are resident in the NIHR-WTCRF, hospital infection control procedures will be strictly enforced and transmission to other humans is extremely unlikely. Participants will be trained in infection control, and they will undertake to practice this after discharge at the conclusion of their residential stay in hospital. During the admission, the potential for environmental shedding of the GMOs will be assessed by measuring colony counts of organisms expired onto a mask worn for 1 hour, and from a 300 L/min air sampler conducted in an environmental chamber at the CRF. This will be done under the guidance of PHE Porton Down scientists, both at intervals during the admission and at each outpatient visit. These data will be reported to the Data Safety and Monitoring Board (DSMB), who will dynamically (re)assess the likelihood of onward transmission of the GMO.

After discharge into the community, it is anticipated that 50% of the volunteers will be carrying one of the GMO strains in the nose and throat and there is the potential for transmission of that GMO to household contacts especially sleeping partners. To monitor this, any persons who share a bedroom with the participants who are inoculated will be (a) asked for informed consent *a priori*, (b) asked to undergo a throat swab prior to inoculation and 2 weeks after inoculation of the participant. If these individuals are found to be carrying *N. lactamica,* they will be offered ciprofloxacin eradication therapy, although it should be reiterated that we do not anticipate any adverse effects in GMO-colonised individuals. In addition, participants will be required to avoid pubs and clubs in the 2 weeks after discharge and to refrain from oral sex, to minimise the chances of disseminating the gene expression construct to *N. gonorrhoeae*.

5. Short description of average environmental conditions (weather, temperature, etc.)

Although we anticipate the majority of volunteers to be resident in Southampton for the duration of the study period, there are no restrictions on the travel of volunteers between visits. The average annual climate data for Southampton is included in Appendix A. Note that during the 5 day admission to the NIHR-WTCRF, the environment will be consistent.

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.

Not applicable, this is the first application to release the GMOs.

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)	Neisseriaceae
(ii)	family name for plants	…
(iii)	genus	*Neisseria*
(iv)	species	*Neisseria meningitidis*
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	*Neisseria meningitidis* (Nmen)

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

The only established interaction of *N. lactamica* with other organisms resident in the nasopharynx is the exclusion of *N. meningitidis* from carriage. This phenomenon requires an individual to be actively colonised with Nlac, that is to say that viable *N. lactamica* can be recovered from throat swabs and/or nasal washes of the individual. The precise mechanisms of this inverse association is still unknown, but is likely to manifest at an ecological level, with the two closely related species competing for the same or extremely similar niche(s). Repeated attempts in our laboratory to identify direct mechanisms of killing of one species by the other have been unsuccessful. Indeed, a recent paper demonstrated co-cultivation of these bacteria in a mixed-species biofilm [36], which corroborates our own, unpublished findings. We aim to shed more light on the impact of experimental challenge with this organism on the nasopharyngeal microbiota, although our preliminary analysis of sample microbiome data suggests the observed replacement phenomenon is exquisite and non-disruptive to other resident bacteria.

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

None known.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?

Yes (.) No (.) Not known (X)

Give details

As stated in Section E.1, although we predict that expression of NadA by strain 4NB1 will lead to a transient colonization advantage that gives way over the longer term to a survival disadvantage, mediated by seroconversion of the colonized individual against the NadA protein; we have yet to

determine this empirically. The epithelial cell receptor for NadA is currently unidentified, and could enable the GMO to persist in a new niche within the nasopharynx. It is important to note that, even in the case of increased persistence in carriage, the GMO will not be able to cause invasive disease because it lacks a polysaccharide capsule, which is the main virulence determinant of the occasionally pathogenic *N. meningitidis*.

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

The GMOs are human-adapted nasopharyngeal commensals with no other biological reservoir, meaning the impact of the release on the environment is negligible. There is the possibility that there will be transmission of the GMO from volunteers to close contacts, especially bedroom sharers, but this is not a cause for concern because the GMO is derived from a commensal bacterium and is incapable of causing disease.

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:

There is evidence of frequent genetic exchange from *N. lactamica* into both *N. meningitidis* and *N. gonorrhoeae*, wherein Nlac serves as a reservoir of alleles for its relatives. Therefore escape of the gene expression constructs from the GMOs is possible. The risk of this is minimized due to the integration of the gene expression construct into the chromosome, meaning there is only one copy present per bacterium. In addition, the construct contains no means to replicate outwith the chromosome, requiring it to become integrated into the genome of another species in order to be maintained. The frequency with which this occurs is influenced by factors such as the composition of the microbiome, the competence of the bacteria that come into contact with the construct, the compatibility of Nlac-donated DNA with the restriction//modification systems present in the recipients and the propensity of the recipient to assimilate exogenous DNA. It is impossible to predict how these factors will align in each inoculated individual, but the overall likelihood of construct dissemination within an individual is assessed to be very low. However, once released, there is the possibility that the GMO will remain in circulation, which means escape of the construct over evolutionary time is inevitable.

(b) from other organisms to the GMO:

Neisseria lactamica is extremely resistant to acquiring new genes through horizontal gene transfer. Although naturally competent, *N. lactamica* is refractory to transformation with DNA from other bacterial species (heterotypic DNA), due to the incompatibility of donated chromosomal DNA. This

incompatibility arises because most other bacteria lack DNA methylases capable of protecting their nucleic acids against the restriction endonuclease activities of Nlac. As a result, most heterotypic DNA taken up by Nlac is degraded before recombination with the chromosome can take place. Indeed, a novel transformation system involving hypermethylated DNA had to be developed in order to derive the GMOs in the first instance. The hypermethylated DNA contains a 5-methyl-cytosine residue in every cytosine position, with the effect of blocking the restriction endonuclease activities of the most potent Nlac enzymes, NlaIII and NlaIV. As such, the likelihood of the GMOs acquiring genetic material from indigenous organisms (i.e. other nasopharyngeal commensals) is minimal.

In support of this, we made a longitudinal analysis of whole genome sequences derived from serial isolates of wild type *N. lactamica* strain Y92-1009, in which we observed only a very few nucleotide changes over time. Most commonly these were changes in the length of homopolymeric nucleotide tracts, meaning the fixed genetic changes we observed over time were attributable to mechanisms of phase variation. There was no evidence for the chromosomal integration of DNA from exogenous sources (e.g. heterotypic DNA), even in isolates that had been carried *in vivo* for 6 months following experimental colonization.

DNA uptake by *N. lactamica*, like the other members of the Neisseriaceae, is biased in favour of nucleic acids containing compatible DNA Uptake Sequences (DUS). Nucleic acids containing DUS are preferentially taken up by *Neisseria* species, and the sequence is overrepresented in the Nlac genome. Nlac and Nmen preferentially take up DNA containing the same DUS, meaning that, if Nlac were ever to assimilate heterotypic DNA from another bacterial species, it would most likely be from Nmen. We were concerned that, in this way, Nmen might serve as a reservoir of genes for the synthesis and surface deposition of polysaccharide capsule, which if acquired by the GMO might transition it from harmless commensal to potential pathogen. We demonstrated experimentally that this was not the case, and that the GMOs are refractory to transformation by chromosomal DNA extracted from Nmen. Briefly, nascent colonies of each GMO were exposed to 1 μg of purified chromosomal DNA, extracted from a mutant derivative of *N. meningitidis* strain MC58, in which the *nadA* gene was insertionally inactivated by the kanamycin-resistance conferring gene, *aphA3* (*ΔnadA:aphA3*). Under laboratory conditions optimised for *Neisseria* transformation, the transformation efficiencies of the GMOs with respect to this gene were *zero* (n = 6). The same purified chromosomal DNA transformed wild type *N. meningitidis* strain MC58 with an average efficiency of 0.06952 transformants/CFU/pmol DNA, demonstrating that the DNA was capable of affecting transformation in compatible strains. It is also important to note that the competence of each GMO was demonstrated through transformation with homotypic DNA (i.e. chromosomal DNA extracted from a strain derived from Y92-1009). Using an equimolar amount of chromosomal DNA purified from an antibiotic resistant mutant derivative of Y92-1009, strain *ΔnlaIII:aphA3*, we observed an average transformation efficiency of 1.88 transformants/CFU/pmol DNA in strain 4NB1. NB: These data demonstrate there is potential for the uptake of DNA from circulating strains of *N. lactamica* by the GMO. However, carriage of *N. lactamica* at pre-screening is one of the exclusion criteria for the study, which should minimise the likelihood of a volunteer co-carrying a potential donor. Even in the event of the GMO becoming transformed with DNA from a co-carried strain of *N. lactamica*, the fact that Nlac is non-pathogenic means that the GMO will not acquire any genes with pathogenic potential.

(d) likely consequences of gene transfer:

Accepting that further dissemination of our Nlac codon-optimised version of *nadA* is possible, it becomes important to consider the impact this will have on recipient organisms:

For those organisms unable to express the gene, perhaps due to a significantly different codon usage bias compared to *N. lactamica*, or the incompatibility of their transcriptional machinery with *Neisseria* promoters, there is likely to be little to no effect on the bacteria themselves. These bacteria will, at worst, act as reservoirs for the *nadA* gene, serving as a potential route of indirect transmission to other (*Neisseria*) species in the future. However, there is no reason why this version of the *nadA* gene is any more likely to be disseminated than naturally occurring alleles in currently circulating strains of *N. meningitidis.* The fact that NadA remains a meningococcal-specific adhesin suggests that the gene is *not* being transmitted between *Neisseria* species and that the gene confers no evolutionary advantage to other members of the genus or species outside of the genus.

For those organisms that are able to express the *nadA* gene, we predict the acquisition of this allele will confer upon the recipient a similar short-term benefit to their capacity for nasopharyngeal colonization as strain 4NB1. Specifically, we predict that NadA expression will allow recipient bacteria to bind to the as-yet unidentified epithelial cell receptor for NadA and potentially adhere in greater numbers to more epithelial cells. However as in the GMO, we predict that seroconversion of the host against the highly immunogenic NadA protein, combined with the fact that the *nadA* gene is under transcriptional control of a constitutively active promoter, will ultimately lead to the adhesin becoming a survival liability and the clearance of inadvertently transformed strains.

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.):

Not applicable.

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

The GMOs are exclusively human nasopharyngeal commensals and do not take part in any biogeochemical processes.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

Throat swabs and nasal washes will be taken at Day 0 (challenge), daily during the 5 day admission, and then at 7, 10, 14, 28 and 90 days post-challenge. Bacteria will be cultured from throat swabs and nasal washes and the GMOs identified through the combination of techniques described in Section E.4.b. Ten individual isolates per participant, per time point will be subcultured and stored for later analyses.

2. Methods for monitoring ecosystem effects

In addition to microbial culture techniques, throat swabs from each participant will also be taken and the contents transferred to FTA cards in order to extract and preserve DNA. This will allow for longitundinal analyses of the oropharyngeal microbiota. In addition, the host cell contents of nasal washes will be analysed by flow cytometry. Together these data will contribute toward a better understanding of what impact the GMOs have on the microbial ecology of the oro-nasopharynx.

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

During initial screening and immediately before inoculation, nasal washes and throat swabs taken from volunteers will be cultured. As described in Section E.4.b, putative *Neisseria* colonies will be identified as *N. lactamica* in the first instance by metabolism of X-gal to form a blue breakdown

product. During this screen, the number of other β-galactosidase positive, non-*Neisseria* organisms will be noted to provide a baseline. If during the course of the study, at any return visit, the number of β-galactosidase positive, non-*Neisseria* colonies cultured from a volunteer should increase from baseline by more than two fold, then representative samples of each β-galactosidase positive species will be cultivated and used to prepare genomic DNA. Each of these representative DNA samples will then be subjected to the same diagnostic PCR as the putative GMO colonies, to determine whether there has been dissemination of the gene expression cassette. The diagnostic PCR exploits the unnaturally close linkage of the *porA* promoter region and the *lacZ* gene, which due to their proximity we assume will be transferred as a single unit. Whilst we acknowledge that the numbers of β-galactosidase positive, non-*Neisseria* species is liable to fluctuate, it is prudent to set a low threshold for initiating this procedure, as allele escape will need to be reported for consideration by the Data Safety Monitoring Board. We acknowledge the limitation of this procedure is that it will only allow us to identify allele escape into a narrow range of other nasopharyngeal commensals, specifically those that can be co-cultured on the media used to sustain the recovered GMOs.

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

Not applicable, GMOs will be isolated from samples taken directly from volunteers (or their close contacts).

5. Duration of the monitoring

The GMOs will be monitored throughout the duration of the study, with specimens regularly collected for storage and downstream analysis. Recovery of the GMOs from inoculated participants is one of the primary outputs from the study, with regard to assessing duration of carriage of the GMO.

Monitoring of the participants during their residential stay in hospital will take place for 5 days post challenge**.** Continuous participant safety monitoring will occur throughout the challenge period through a combination of daily clinical review and monitoring of symptoms in an electronic diary. All study participants will agree to have 24-hour contact with study staff during the 90 days post challenge and to be able to ensure that they are contactable by mobile phone for the duration of the challenge period until the end of the study. An independent Data Safety Monitoring Board (DSMB) will be established prior to the start of the study. The DSMB will be appointed to provide real-time oversight of safety and trial conduct. The DSMB will have access to data and, if required, will monitor these data and make recommendations to the study investigators on whether there are any ethical or safety reasons why the study should not continue. The DSMB will also be notified immediately if the study team have any concerns regarding the safety of a participant or the general public. The outcome of each DSMB review will be communicated directly to the study investigators and documentation of all reviews will be kept in the trial master file. The Chair of the DSMB will also be contacted for advice when the Chief Investigator feels independent advice or review is required.

6. Frequency of the monitoring

Throat swabs and nasal washes will be taken at Day 0 (challenge), daily during the 5 day admission, and then at 7, 10, 14, 28 and 90 days post-challenge.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

After the volunteers for that day have been challenged, the room will be cleaned following NHS infection control guidelines.

2. Post-release treatment of the GMOs

A small aliquot (30µl) of the residual inoculum from each participant will be used to perform a viability count. Any remainder will be inactivated with excess 2 % Virkon solution, which kills *N. lactamica* (and the GMO strains) within 2 minutes. Virkon solutions so-used will be left overnight before proper disposal.

3. (a) Type and amount of waste generated

Relevant clinical waste (blood/saliva/nasal swab samples, tissues, sharps, syringes, disposable clothing, gloves, gowns, masks and aprons).
Laboratory waste (including but not limited to: plastic ware, microbiological waste (agar plates/blood culture bottles, filters), gloves, disposable clothing, paper towels, and clinical samples as described above).

The amount of waste will be typical for the clinical site and laboratory operations amounting to a few clinical waste bags and bins per day. The amount of expected waste will be managed by standard operating procedures currently in place at the site.

3. (b) Treatment of waste

Waste will be treated according to site standard operating procedures (SOPs) for handling GMO and potentially infectious GMO waste. All GMO activity at site will be inactivated by autoclaving prior to disposal and removal from the site. All associated procedures have been validated, site autoclaves are accredited annually with contracts in place for regular equipment servicing and maintenance. Post-release GMO shed in the nasopharyngeal samples of study participants will be eliminated according to the SOPs described above.

J. Information on emergency response plans

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

Within the Southampton NIHR Clinical Research Facility strict precautions are in place to avoid the spread of the GMO from the study participant to others. If the participant sneezes onto a surface at any time, this will be treated as any spillage per SOPs.
Public Health England (PHE) investigators are involved in this study, and the relevant section of the organisation (e.g. the Meningococcal Reference Unit) will be informed that it is underway. Public Health Southampton (now part of Southampton City Council) will be informed of all participants who have been challenged with the GMOs. The participants' GP will also be informed. In addition, any unexpected occurrence of disease in participants will be notified to the data and safety monitoring board. If the DSMB considers the event to be causally related to the GM, the study will be stopped, per protocol and this information will be passed on immediately to PHE and Public Health Southampton as well as the GP.

Unexpected spread within the community would be detected if (a) microbiology laboratories reported to Public Health England that an unusually high number of *N.lactamica* isolates were being detected in routine throat swabs taken from children or adults with suspected sepsis, or (b) disease occurred in individuals other than participants in the study. Of these, (a) would require microbiology laboratories to be made aware of the study and to be asked not to discard non-pathogenic *Neisseria* cultured from routine swabs during the period of the study, and (b) is extremely unlikely because the GMO is non-capsulate and non-virulent in a recognised animal model of *Neisseria* disease, but would be detected in routine speciation of invasive isolates of a patient with sepsis. *Neisseria lactamica* would be such an unusual cause of disease that PHE

surveillance would track the occurrence back to this study, via the PHE Meningococcal Reference Unit, who are aware of this study, and hold stock of the wild type organism. If (a) occurs it is unlikely that action would be taken in the absence of any disease caused by the GMO, but public health authorities would have the option of using the same strategy that is used in outbreaks of meningococcal disease, i.e. single dose ciprofloxacin to clear carriage in close contacts or alternatively to vaccinate with the NadA-containing vaccine Bexsero, which has been shown to protect against the occurrence of invasive disease in the case of *N.meningitidis*. If (b) occurs then index cases would be treated with ceftriaxone and contacts would have prophylaxis with ciprofloxacin. Bexsero vaccine would be available if public health authorities deemed it necessary to protect larger populations.

To detect potential disease in participants, they will be instructed to notify the study team of any serious adverse events/reactions following administration of the GMO. All participants agree to have 24-hour contact with study staff during the 90 days post challenge and to be able to ensure that they, or a friend/family member are contactable by mobile phone for the duration of the challenge period until antibiotic completion. A physician from the clinical team will be on-call 24 hours.
Participants will be issued with a Medic Alert-type card containing information including the antibiotic sensitivity of the GMO, study doctor contact details and instruction for the research team to be contacted immediately in the event of illness/accident.
Potential participants with known antibiotic hypersensitivity or allergy to either of the first-line antibiotics (ciprofloxacin, ceftriaxone) will be excluded from the study.
There are provisions within the protocol and site facilities to allow for admissions of participants as inpatients to University Hospital Southampton in cases of sepsis arising in participants during the study period.

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

Contaminated areas such as laboratory or clinical area spills may be decontaminated using 2 % Virkon solution and a minimum of 2 minutes of contact time. Clinical carriage can be eradicated with single dose oral ciprofloxacin.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread

Not applicable.

4. Plans for protecting human health and the environment in the event of an undesirable effect

If there is detection of asymptomatic carriage of the GMO in the community apparently unconnected with the participant it is unlikely that any action would be taken by public health authorities, but public health authorities would have the option of using the same strategy that is used in outbreaks of meningococcal disease, i.e. single dose ciprofloxacin to clear carriage in close contacts or alternatively (if there is disease caused by the GMO) to vaccinate with the NadA-containing vaccine Bexsero which has been shown to protect against the occurrence of invasive disease in the case of *N.meningitidis*. In the extremely unlikely event of disease occurring in the participants, or in an unconnected person, they would be treated with the intravenous antibiotic ceftriaxone and contacts would have prophylaxis with the oral antibiotic ciprofloxacin, as per clinical guidelines for meningococcal disease. Bexsero vaccine, which contains the same NadA antigen as the GMO, would be available if public health authorities deemed it necessary to protect larger populations.
The environment will be unaffected because this organism exclusively colonises humans.

Appendix A:

Table 1 – Annual average climate data (1981-2010): Southampton, UK.

Climate data for Southampton, elevation 3 metres (9.8 feet), 1981–2010													
Month	**Jan**	**Feb**	**Mar**	**Apr**	**May**	**Jun**	**Jul**	**Aug**	**Sep**	**Oct**	**Nov**	**Dec**	**Year**
Average high °C (°F)	8.4 (47.1)	8.6 (47.5)	11.1 (52)	14.0 (57.2)	17.5 (63.5)	20.2 (68.4)	22.4 (72.3)	22.3 (72.1)	19.8 (67.6)	15.6 (60.1)	11.7 (53.1)	8.9 (48)	15.1 (59.2)
Average low °C (°F)	2.9 (37.2)	2.6 (36.7)	4.1 (39.4)	5.7 (42.3)	9.0 (48.2)	11.7 (53.1)	13.7 (56.7)	13.7 (56.7)	11.4 (52.5)	8.9 (48)	5.4 (41.7)	3.2 (37.8)	7.7 (45.9)
Average rainfall mm (inches)	81.4 (3.205)	58.3 (2.295)	60.0 (2.362)	50.7 (1.996)	49.0 (1.929)	50.4 (1.984)	42.0 (1.654)	50.4 (1.984)	60.4 (2.378)	93.8 (3.693)	94.0 (3.701)	89.2 (3.512)	779.4 (30.685)
Average rainy days (≥ 1.0 mm)	12.2	9.2	10.1	8.8	8.2	7.7	7.4	7.7	8.7	11.5	11.5	11.8	114.7
Mean monthly sunshine hours	63.3	84.4	118.3	179.8	212.1	211.2	221.8	207.7	148.1	113.0	76.6	52.9	1,689.3
Source #1: Met Office (normals)[78]													
Source #2: Calculated from Met Office Data[79]													