Part B: Information about the release application to be included on the public register

B1 The name and address of the applicant

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B2 A general description of the genetically modified organisms in relation to which the application is being made

The organism is *Neisseria lactamica*, a bacterium which lives only in the nose and throat of humans where it resides as a colonising harmless commensal. This bacterium is carried most commonly by infants and toddlers and becomes less common in older children and adults. It is from the same genus as a similar bacterium, *Neisseria meningitidis*. The latter organism also lives in the nose and throat of humans and transmits between people in very close contact. *Neisseria meningitidis* carriage is mostly harmless, but in a very small proportion of people who are carriers, the bacterium may enter the bloodstream and cause meningococcal disease, including meningitis. Of particular note there is an inverse relationship between carriage of *Neisseria lactamica* and carriage of *Neisseria meningitidis*, and in a previous experiment in which we deliberately infected 300 students with *Neisseria lactamica*, it was shown that subsequent natural infection with *Neisseria meningitidis* was significantly inhibited over the course of the University year. The **genetic modification** will result in *Neisseria lactamica* having an inserted gene that results in expression of a protein called NadA. NadA is a protein made naturally by *Neisseria meningitidis*, and is included in the new meningitis B vaccine (Bexsero). To

do this, the gene *nadA* has been inserted into the bacterial chromosome of *Neisseria lactamica*. No antibiotic selection marker has been included, but instead a gene native to *Neisseria lactamica* (*lacZ*) has been moved to a new location in the chromosome. A second GMO has been made which is a `control` organism, which has gone through the same process except that it does not contain the *nadA* gene.

B3 The location at which the genetically modified organisms are proposed to be released

The release will take place in the Southampton NIHR Wellcome Trust Clinical Research Facility, at University Hospital Southampton, Tremona Rd, Southampton, Grid Reference SU397149.

B4 The purpose for which the genetically modified organisms are proposed to be released (including any future use to which they are intended to be put).

Purpose of the deliberate release: The ultimate purpose is to improve the way we protect humans from severe bacterial diseases including meningitis. In this clinical study we propose to utilise an established human challenge model for inoculation of human volunteers with wild type *Neisseria lactamica* to further study host-bacterial interactions. In the model, volunteers are infected in the nose with a low dose (10,000) of *Neisseria lactamica*. We will investigate whether experimental colonisation by the NadA expressing GMO results in immunity directed specifically against NadA. This information will be used to (a) understand how mucosal immunity to meningococcal antigens such as NadA (a vaccine component) develops (b) to inform vaccine development by permitting the development of a colonisation model in which the colonisation-protection efficacy of vaccines containing NadA can be tested, and (c) develop potential `bacterial medicines` in which genetically modified commensals are used to deliver immunising or microbiome-modifying gene products into the nasopharynx for health benefit.
The proposed study will follow a protocol entitled `Experimental challenge of the human nasopharynx with recombinant *Neisseria lactamica* expressing the meningococcal type V autotransporter protein Neisseria Adhesin A (NadA)`
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.

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 *N.lactamica* 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 *N.lactamica*-colonised volunteers.

Because carriage of *N.meningitidis* is pre-requisite for the development of meningococcal disease, reduction of *N.meningitidis* 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.

B5 The intended dates of the release.

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).

B6 The environmental risk assessment.

Neisseria lactamica`s only natural habitat is the human nose and throat, so there will be no environmental impact.

B7 The methods and plans for monitoring the genetically modified organisms and for responding to an emergency.

The GMO *N.lactamica* is very different to the meningitis-associated bacterium because it is not surrounded by a sugar capsule, which allows *N.meningitidis* to penetrate the blood stream and cause disease. It is extremely unlikely that the GMO will be capable of causing disease; this has been supported using experiments in mice. The GMO is fully sensitive to all the antibiotics used to treat *N.meningitidis* disease so in the unlikely event of any disease, there is an available strategy for control.

Notwithstanding this, close monitoring will take place for the duration of the clinical study. All study participants will have follow-up visits up to 90 days post challenge with nasopharyngeal sampling at regular intervals as outpatients.

Following challenge with the GMO, participants will be monitored daily as inpatients in the hospital facility for the first 5 days post-challenge. This is because it will be the first time that the GMO has been used to infect humans, and if, unexpectedly, the GMO is capable of causing disease, we expect this will happen in the first few days after colonization of the nose and throat, because this is what happens when people are naturally infected with the bacterium *Neisseria meningitidis*. 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.

Neisseria meningitidis is known to transmit to close contacts of those carrying the organism, particularly those who share households. There is much less information on natural transmission of *N. lactamica*, but we expect that household members, in particular bedroom-sharers of the participants, will be the likeliest persons to be colonised by onward transmission of the GMO. However, transmission beyond the household setting could be possible. Therefore the action we propose is to limit as much as is reasonable, onward transmission into the community. To minimise the community spread of the GMO, strict infection control procedures will be enforced during the inpatient stay of the participants in the hospital. 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.

Following discharge, the participants will be returning to their community. If they share a bedroom, the bedroom-sharer will be required to provide informed consent, and a baseline swab will be taken, plus a throat swab 14 days after discharge of the participant from hospital. The bedroom sharer will also be trained in infection control practice, but if they become colonised at 14 days after discharge of the participant, they will be treated with an antibiotic ciprofloxacin, which is commonly used to clear carriage of *N.meningitidis*, and is an antibiotic to which the GMO is fully sensitive. The participant will be required to avoid pubs and clubs in the 2 weeks after discharge.

One of the exclusion criteria is that participants must be persons who have no contact with immunosuppressed individuals. The purpose of this is to limit, as much as possible, the possibility of carriage of the GMO by anyone who might be vulnerable to infections in which *N. lactamica* might become involved.

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 notified. In addition, any unexpected occurrence of disease in participants will be notified to the data and safety monitoring board (DSMB). 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 of the GMO 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 caused by the GMO occurred in individuals – whether study participants or members of the general public. 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 the antibiotic ceftriaxone (commonly used to treat bacterial disease in hospitals) and contacts would have prophylaxis with the antibiotic ciprofloxacin. Bexsero vaccine would be available if public health authorities deemed it necessary to protect larger populations.