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 that has been modified is *Neisseria lactamica*, a bacterium which lives only in the nose and throat of humans, where it resides as a harmless colonising commensal. This bacterium is carried most commonly by infants and toddlers and becomes less common in older children and adults. It is closely related to 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 unmodified *Neisseria lactamica*, it was shown that subsequent natural infection with *Neisseria meningitidis* was significantly inhibited over the course of the University year.

Neisseria lactamica has been genetically modified by inserting two genes into its chromosome that allow the bacteria to make two additional proteins, called Neisseria Adhesin A (**NadA**) and Factor H-binding protein (**FHbp**). Both are proteins made naturally by *Neisseria meningitidis*, and are included in the meningitis B vaccines (Bexsero, which contains both NadA and one type of FHbp; and Trumenba, which contains 2 different types of FHbp). Because the shape of the FHbp protein varies between strains of *Neisseria meningitidis*, and because the aim of the experiment is to increase the degree to which *Neisseria lactamica* "looks like" *Neisseria meningitidis* to the immune system, it was necessary to make 4 different strains of genetically modified *Neisseria lactamica*. Each of these four strains is able to make one different variant of FHbp and all the strains make the same variant of NadA. The four strains will be mixed together in equal numbers before being used to infect study participants. The name of this mixture of bacterial strains is called **4xrNlac**. This mixture of strains is therefore representative of the diversity of natural FHbp and it is thought this will improve the amount of protection that infection with GM-*N. lactamica* will hopefully generate against subsequent, natural infection with *Neisseria meningitidis* (see B4, below).

Importantly**,** we do not expect the mixture of GM-*N. lactamica* strains to cause any symptoms or disease. The main difference between *N. lactamica* and *N. meningitidis* is the ability of *N. meningitidis* to make a protective capsule of sugars on its surface. This capsule is essentially what allows *N. meningitidis* to survive in the bloodstream and cause disease. Neither naturally occurring, nor the genetically modified strains of *N. lactamica* make such a capsule and are therefore unable to cause disease. Note that the NadA protein only helps *N. meningitidis* stick to cells, it does not allow *N. meningitidis* to survive in the bloodstream or to invade cells, and therefore cannot make the GM-*N. lactamica* strains capable of causing disease. When made by *N. meningitidis* during an infection*,* the FHbp proteins bind to the human complement protein, **Factor H**, which is a protein in blood that effectively stops the immune system from attacking the body. By binding Factor H, the bacterium therefore attempts to stop the immune system from attacking it. FHbp is not as important as capsule in protecting *N. meningitidis* in the bloodstream, but it does make a contribution to the survival of the bacteria in this environment. A legitimate concern might therefore be that by adding FHbp to GM-*N. lactamica*, we might inadvertently increase the risk to our participants. Importantly therefore, in the GM-*N. lactamica* strains, each of the genes coding for FHbp have been changed in a very small way, such that the proteins are no longer able to bind to Factor H and are therefore

unable to interfere with a normal immune response. Note that there is nothing else about FHbp that could make the GM-*N. lactamica* strains capable of causing disease.

We have demonstrated that all four GM-*N. lactamica* strains in the 4xrNlac challenge agent are efficiently killed by normal human plasma, that they are susceptible to routinely used antibiotics and that the genetic modifications have not changed the lifestyle or behaviour of the organisms. We have conducted extensive testing to show that the genetic modification has not changed anything else about the bacteria that might cause it to suddenly be able to survive in the bloodstream and cause disease. The genetic modification of *N. lactamica* will therefore confer no increased ability to cause disease in humans in comparison to harmless, naturally-occurring *N. lactamica*.

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

The release will take place in the Southampton NIHR Clinical Research Facility, at University Hospital Southampton, Tremona Rd, Southampton **SO16 6YD**.

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 aim of the programme of work is to improve the way we protect humans from severe bacterial diseases, including the rapidly lethal sepsis syndrome, invasive meningococcal disease (IMD). To achieve this, we need to inform the rational design of future vaccines, such that they generate sterilising immunity that prevents people from carrying these bacteria in their bodies without any disease symptoms (known as asymptomatic carriage). By preventing asymptomatic carriage, the frequency with which bacteria are transmitted between members of the community will be reduced, which in turn decreases the likelihood of a person susceptible to severe disease becoming infected. To inform the rational design of future vaccines, it is essential to understand whether and by what means we can establish an immunological state, or “phenotype”, which prevents asymptomatic carriage, so that later on we can design therapies that recapitulate this state. We intend to release the genetically modified strains of *Neisseria lactamica* in two clinical studies, collectively titled “The GM-Nlac Study”, with the aim of establishing whether, after becoming asymptomatically infected with one or more strains of genetically modified *Neisseria lactamica*, a person becomes immune to asymptomatic carriage of the same genetically modified *Neisseria lactamica* a second time. If this proves to be the case, and infection with genetically

modified *Neisseria lactamica* leads to a “protected against carriage of genetically modified *Neisseria lactamica*” phenotype, then there is the potential for the same mixture of bacteria to also be useful in preventing asymptomatic carriage of *Neisseria meningitidis*. This is on the basis that *Neisseria lactamica* and *Neisseria meningitidis* share many surface structures, and the genetic modification serves to increase this similarity. It is therefore plausible that the genetically modified *Neisseria lactamica* might ‘fool’ the immune system into simultaneously establishing a state where asymptomatic carriage of *Neisseria meningitidis* is impossible. The exact immunological mechanisms that underpin any ‘protected against carriage’ phenotype are currently unknown and it is possible that the same phenotype is generated by more than one different combination of immune responses (i.e. different ‘fingerprints of protection’). By taking samples from participants across the duration of the study, we aim to deconvolute these immune responses and identify those which, either in isolation or in combination, afford the induced immunity to asymptomatic carriage.

In the proposed clinical studies, we will administer a relatively low dose (400,000 CFU) of a mixture of 4 different genetically modified *Neisseria lactamica* strains (4xrNlac) into the noses of healthy participants (this is known as ‘challenge’), and allow the bacteria to live asymptomatically in the nose and throat of these participants for a period of time. During the studies, participants will be closely monitored for carriage of 4xrNlac, and for any symptoms or illness. We will also assess an array of immune responses specifically directed against the genetically modified *Neisseria lactamica.* In the first study – “The GM-Nlac – Pilot” study, ten participants will be challenged with 4xrNlac, after which they will be followed up for a period of 28 days. Carriage with 4xrNlac will be cleared with oral antibiotics at the end of the study. This pilot study aims to ensure that we can effectively and safely induce carriage with 4xrNlac. Following completion of this study, “The GM-Nlac – Main” study will enrol up to 62 participants who will be randomised into two groups, one group will be challenged with 4xrNlac, whilst the other will receive a control inoculum that does not contain any bacteria. Following challenge they will be monitored for a period of 56 days and we will collect samples from their noses and throats at different time points. All participants will then receive antibiotics, which will clear the bacteria from carriage if they are present. There will then be a second challenge at which time all participants will receive 4xrNlac, with a further 56 day follow up period and an identical regimen of sampling. After this second period all participants will be given a second dose of oral antibiotics to clear carriage at the end of the study. The aim of this study is to investigate whether carriage of genetically modified *Neisseria lactamica* induces a ‘protected against carriage’ phenotype, in which participants who received 4xrNlac at the first challenge were then protected against carrying exactly the same strains of genetically modified *Neisseria lactamica* after the second challenge, in comparison to those participants who received the control (bacteria-free) inoculum at the first challenge. If we observe a ‘protected against carriage’ phenotype in our participants, we will use their samples to measure a variety of immune responses and attempt to work out what makes up a particular

participant's 'fingerprint of protection'.

Potential future uses of the GMO: One immune response that might contribute to certain 'fingerprints of protection' is the generation of antibodies that specifically target the proteins present on the genetically modified *Neisseria lactamica*. Certain antibodies, such as those made in response to proteins included in vaccines, are able to target bacteria for killing by other parts of the immune system. These are known as 'bactericidal antibodies'. Because the genetically modified *Neisseria lactamica* strains each make 2 vaccine proteins found in meningitis B vaccines, there is a chance that participants who carry the bacteria after challenge will make bactericidal antibodies targeting NadA and FHbp. Because 4xrNlac contains 4 strains, which collectively represent the diversity seen in the structure of natural FHbp, there is a chance that a given participant will generate bactericidal antibodies against more than one type of FHbp. If participants generate these bactericidal antibodies at all, they might make enough of them that their blood becomes inhospitable towards invading strains of *Neisseria meningitidis* that also make the same or similar NadA and/or FHbp proteins. Therefore, participants with enough bactericidal antibodies targeting these vaccine proteins in their blood would become protected against invasive meningococcal disease that these strains of *Neisseria meningitidis* might otherwise cause. Because we know there is a certain amount of bactericidal antibodies needed in the bloodstream to protect a person against invasive meningococcal disease, if we find 4xrNlac is able to generate this type of response across lots of participants, there is potentially a future use for it as a new type of vaccine to prevent invasive meningococcal disease.

If we observe a 'protected against carriage with genetically modified *Neisseria lactamica*' phenotype after participants receive a second dose of 4xrNlac, then we would need to design a new study to test whether we can use 4xrNlac to generate a 'protected against carriage with *Neisseria meningitidis*' phenotype. This would need to be a much larger study, known as a 'field trial', where we would give participants either a dose of 4xrNlac or a bacteria-free control, and then monitor them over time to see if they acquire asymptomatic carriage of strains of *Neisseria meningitidis* from their communities.

B5 The intended dates of the release.

"The GM-Nlac – Pilot" study is expected to commence on the 1st of February 2026 (pending all necessary approvals) and will run for approximately 3 months (with an expected completion date of 30th April 2026). "The GM-Nlac – Main" study is expected to commence on the 1st November 2026 (pending all necessary approvals) and will run until study completion, anticipated to be 31st May 2031. We are therefore seeking consent to conduct deliberate release of 4xrNlac from: 1st February 2026 to 31st May 2031 inclusive.

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.

Following challenge with 4xrNlac, we expect one or more of the GM strains to survive and multiply in the nose and throats of participants, in other words they will become carriers. This carriage may continue from the time of challenge until they receive antibiotics to clear carriage, that is for 28 days in the Pilot study and 56 days after each challenge in the Main study. During this period, participants will be monitored at regular follow up visits. We will take throat and nose samples and blood tests to assess for carriage of the strains and their immune responses.

It is extremely unlikely that the GM-*N. lactamica* strains could cause any symptoms or disease, as explained above, specifically because they lack the capsule required to survive in the bloodstream. However, we will monitor participants for any signs or symptoms developing during the study. We will routinely collect this information at each follow up visit, but in addition, participants will have a 24 hour telephone number to contact the study team and will be reviewed at additional visits if required. If symptoms develop that we are concerned may be related to the GM-*N. lactamica* strains then we will treat these with routinely used antibiotics, to which the strains are known to be fully susceptible. At the end of each challenge period all participants will receive antibiotics to clear carriage of the GM-*N. lactamica* strains.

N. lactamica could potentially be transmitted from person-to-person via respiratory droplets, which requires close contact. However, we have never observed shedding or transmission of *N. lactamica* from carriers to their contacts. Extrapolating what we know about transmission from *N. meningitidis*, the highest chance of transmission would likely be between household contacts, and in particular those that share a bedroom with a carrier. In order to look for transmission we will enrol individuals who share a bedroom with participants as 'contact participants'. We will only enrol participants when both the challenge and any contact participant meet specific elibility criteria. Potential participants who have household or occupational contact with young children or those with immune system problems will be excluded. All challenge and contact participants will be required to abide by strict infection control rules throughout the study. These criteria and rules aim to prevent transmission to any other individuals, particularly those who might be more vulnerable. During the study, contact participants will also have access to the 24 hour telephone number and will be reviewed if they develop any symptoms. At the end of the study, a throat swab will be taken to look for transmission of the GM-*N. lactamica* strains. If this is

positive, i.e. any of the strains have been transmitted, then they will be given antibiotics to clear this carriage.

These steps will minimise the possibility of transmission to any other contacts and into the wider community. However, we recognise that it remains possible that further transmission might occur. In this event, the GM-*N. lactamica* strains would not be expected to cause any symptoms or disease. However, as the strains are susceptible to routinely used antibiotics, any disease could be treated effectively with standard medical care if required. In the extremely unlikely event of a public health concern arising, public health authorities would have the option of using the same strategy that is used in outbreaks of meningococcal disease, i.e. oral antibiotics to clear carriage or alternatively to vaccinate with meningococcal vaccine Bexsero.

As *N. lactamica* does not spread or survive other than in the noses and throats of humans, there is no risk of transmission to any other species or survival in the environment.