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

B1 The name and address of the applicant

Programme Lead/ Lead Scientist	Clinical Science -Lecturer in Vaccinology and Infectious Disease Centre for Tropical Infectious Diseases Liverpool School of Tropical Medicine Accelerator Building 3rd Floor 1 Daulby Street, Liverpool L7 8XZ
Chief Investigator - Clinician	Senior Clinical Lecturer Liverpool School of Tropical Medicine (as above)
Collaborator -Lead for Genetic Modification	University College London Respiratory Rayne Building 5 University Street London WC1E 6JF
Senior Clinical Research Associate	Liverpool School of Tropical Medicine (as above)

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

Streptococcus pneumoniae (S. pneumoniae)
Capsular serotype 6B strain BHN418
Serotype 6B capsule – Quellung reaction with type specific antisera

We will use genetically modified strains of the above 6B strain containing deletions of a limited number of genes (two or three); hence the GM modified strains will be 99.8 – 99.9% genetically identical to the parental 6B *S. pneumoniae* strain. The mutations delete genes required for *S. pneumoniae* virulence, making the mutant strains much less likely to cause disease than the parental wild-type strain. As *S. pneumoniae* are naturally transformable, two or more virulence genes will be mutated to minimise the chance of revertants developing. The mutations will result in mutant strains that have demonstrated reduced virulence in animal models of systemic disease. We anticipate they will still able to colonise the nasopharynx; although this is likely to be for a shorter period of time than the wild-type parental strain.

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

The release will take place in the Accelerator Research Clinic, 3rd Floor Accelerator Building Liverpool School of Tropical Medicine 1 Daulby Street Liverpool L7 8XZ. Participants will then immediately return to the wider community with follow up visits at the clinic.
Grid Reference: SJ 35782 90814

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).

The purpose is to conduct a clinical trial in healthy volunteers to determine whether nasopharyngeal colonisation of adults with the mutant *S. pneumoniae* increases serotype-independent protective adaptive immunity.

Nasopharyngeal colonisation of adults with wild-type *S. pneumoniae* increases serotype-independent protective adaptive immunity but could on occasion cause severe disease. A safer alternative may be to use intranasal administration of live mutant *S. pneumoniae* strains that are unable to cause serious infections due to deletions of genes encoding virulence factors but which still retain their ability to boost adaptive immunity. We will use an established EHPC model of pneumococcal carriage model (EHPC) to assess the human immune response to colonisation with the mutant pneumococcus bacteria. This model has been used in over 1000 healthy adults in Liverpool for the past nine years safely using wild-type *S. pneumoniae* with no cases of active infection or related serious adverse effects. Safety data will be assessed in volunteers at follow up visits and they will be screened to minimize the risk of invasive illness. In future an attenuated strain may be administered to subjects who are more susceptible to *S. pneumoniae*.

Experimental Human Pneumococcal Challenge Model (EHPC) is has been developed by this team to assess the human immune response to colonisation with pneumococcus bacteria.

Primary Objective: To test whether nasal administration of attenuated *S. pneumoniae* strains prevents future colonisation with wild type *S. pneumoniae* using the EHPC model.

Target is 108 completed participants of young healthy volunteers aged 18 – 50 years. We may recruit up to 150 participants to allow for drop out.

Intervention: inoculated nasally with either an attenuated strain type I or type II **Control:** 6 B wild type S*. pneumoniae* or a mock inoculation (saline).

Follow up: samples collected include nasal wash, nasal fluid, nasal cells and blood samples over the next six weeks. A sub-group will have a bronchoalveolar lavage for immunology data.

Challenge: After a six month period each volunteer will be challenged (inoculated nasally) with the wild type 6B *S. pneumoniae*. This will determine whether the immune response to previous colonisation with *S. pneumoniae* mutants (reduced in virulence) prevents subsequent colonisation with the wild type 6B *S. pneumoniae*.

Outcome: the immune response to *S. pneumoniae* colonisation will be assessed using conventional tests of antibody and white cell responses. Results will be compared for before and after colonisation.

In future: ultimately, these data could potentially be used to inform future development of vaccines to prevent *S. pneumoniae* lung infections.

B5 The intended dates of the release.

The intended start date of the release is estimated as September 2018 subject to regulatory and governance. The trial and participant follow up we aim to complete by June 2020 however we may continue until June 2022 for example to allow for replacement of participants who do not complete their follow up or due to recruitment delays.

B6 The environmental risk assessment.

Not applicable – no environmental reservoir.

S. pneumoniae is only found as a human oropharynx and/or nasopharyngeal commensal; there is no environmental reservoir and *S. pneumoniae* is not a commensal of non-human mammalian species, with the exception of horses which are colonised by a subset of *S. pneumoniae* strains that do not include the 6B strain BHN418.

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

Monitoring the GMO: Stocks purity will be assessed in-house by culturing and results will be confirmed by DNA sequencing (Sanger Institute). Also, serotyping and penicillin sensitivity will firstly be carried out in our laboratory

and then will be confirmed in a reference laboratory (Public Health England). Both DNA sequencing, serotyping and antibiotic profile are key elements of the pneumococcal strains characterisation.

Laboratory monitoring: Bacteriological safety of attenuated strains: sequencing and in vitro testing of bacteria recovered to ensure genome and phenotypic stability.

The GMOs that are cultured from nasal wash samples from volunteers can be easily confirmed to be the test and control strains through standard diagnostic microbiology techniques. The combination of the microbiological and molecular methods described above enables the detection and identification of both parent and attenuated strain with 100% accuracy.

Nasal wash samples will be processed using our published protocol (Gritzfeld, Jove: https://www.jove.com/video/50115/experimental-human-pneumococcal-carriage) and will be plated on blood agar plate supplemented with gentamycin or selective antibiotic (spectinomycin / kanamycin). The latter will allow growth of the attenuated strain but not the wild type parent strain, as it is only included in the antibiotic resistance cassette used for gene replacement. Following overnight incubation at 37°C in 5% CO2, alpha-haemolytic, draughtsman shaped colonies will be sub-cultured for optochin sensitivity and the serotype will be tested using a latex agglutination kit (Staten Serum Institute). A multiplex PCR targeting on lytA and the antibiotic resistant gene (or knocked-out genes) will be used as a verification step of the detection of the mutant strain.

Response to an Emergency: A safety protocol is established for the conduct of the EHPC model. Over a period of nine years over 1000 EHPC healthy adult participants have been inoculated with wild type *S. pneumoniae* without any related serious adverse events. These attenuated strains are anticipated to be less virulent therefore less likely to cause serious side effects; they will probably also have lower carriage rates based on pre-clinical data.

Participants are screened initially to exclude those at higher risk of infection or their close contacts. Safety information is provided and guidance on contacting the research team (a doctor is available 24/7) and/or their health care provider. Prior to commencing the trial the strain is tested for sensitivity to antibiotics (amoxicillin) and participants are provided with a supply in the event of early symptoms or to clear/minimize carriage prior to challenge.

Carriage and safety reports are circulated to the trial management group weekly. The Trial Steering Group and/or Independent Data Safety Monitoring Committee will be contacted by the Chief Investigator in the event of any change to anticipated carriage rates or incidence of symptoms or serious

adverse events classed as possibly related as defined by National Research Ethics Service http://www.hra.nhs.uk/documents/2015/06/safety-progress-reports-procedural-table-non-ctimps.pdf . The first groups inoculated will be limited to 5 – 10 participants to review the carriage and safety data. Carriage status is reported weekly for review by both the clinical and laboratory team. Carriage will be monitored and compared to anticipated carriage rates found in previous EHPC studies of wild type *S. pneumoniae.* Symptoms are monitored daily for the first week then at each clinic visit.

The research team are trained in the EHPC model and the requirements for preparing and disposal of the inoculum and other clinical waste consistent with EHPC and Liverpool School of Tropical Medicine Standard Operating Procedures. Clinical SOP's are consistent with NHS standards for the handling and disposal of clinical waste.

Brief Summary of Safety Protocol	
Screening	Excludes participants more vulnerable to infection or their close contacts
Participant Information	A formal presentation supported by a Safety Information Sheet Advised to report symptoms or adverse events during participation
Pre-inoculation	The organism will be tested to confirm sensitivity to antibiotics (Amoxicillin) these are provided to avoid delay in treatment.
Post - inoculation	Participants report their temperature and monitor symptoms daily post inoculation for 3 days. Participants provide details of a close contact A supply of antibiotics is provided to treat early sign of symptoms without delay or to clear/ reduce carriage.
Follow up	Monitor carriage by nasal wash over six weeks post inoculation Weekly carriage and safety reports to the Trial Management Group
Access to health care	GP notified of participation in the trial Research Team doctors are available 24 hours 7 days a week for advice and for triggered assessments during weekdays.
Withdrawn	If a participant or their close contacts circumstances change then they may be withdrawn from the study at any time and if required commence antibiotics.
Oversight	An independent Data Safety Monitoring Committee monitor the safety aspects of the trial.