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

B1 The name and address of the applicant

Oxford Vaccine Group, University of Oxford
Centre for Clinical Vaccinology and Tropical Medicine (CCVTM)
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Oxford
OX3 7LE

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

Description of the GMO

The GMO is an isogenic mutant of a wild-type *Salmonella* Typhi (*S*.Typhi) Quailes strain. The purpose of the genetic modification is to construct a modified *S*. Typhi Quailes strain carrying the deletion of the *cdtB*, *pltA*, and *pltB* genes, to generate a typhoid toxin-deficient *S*. Typhi Quailes strain (SB6000). The GMO will be used to investigate the 'bottlenecks' hypothesis in the pathogenesis of enteric fever using a controlled human infection model. Typhoid fever is caused by infection with the bacterium *S*. Typhi.

Description of the application
The primary objective of the study is to investigate the nature of the bacteraemia that results when a combination of two *Salmonella* Typhi strains are given using a human challenge model of infection. The primary objective of the proposed study is to investigate the nature of the bacteraemia that results when a combination of two *Salmonella* Typhi strains are given using a human challenge model of infection. The primary outcome will be to determine the proportion of participants who develop bacteraemia post challenge with either wild type *Salmonella* Typhi Quailes strain (WT) or a typhoid toxin-deficient isogenic mutant of *Salmonella* Typhi Quailes strain (named SB6000) or a combination of both strains.

The GMO to be released is intended for use in healthy adults aged 18-60 years. The Oxford Vaccine Group (University of Oxford, UK) has been undertaking controlled human challenge studies using *S.* Typhi and *S.* Paratyphi A since 2010.

Inoculation with many microorganisms is often sufficient to cause a bloodstream infection in a susceptible host, typically inoculation with a single microorganism is not. There are two principle theories as to how bacteraemia arises. The first is independent action whereby the bacteraemia that results from bacterial inoculation is derived from a single founder organism ('the bottleneck hypothesis'). Each organism has a chance of being that founder organism. A greater number of organisms make bacteraemia more likely. The second theory is synergy whereby there is cooperation between bacteria, multiple bacteria traverse the barrier to infection and therefore the bacteraemia is composed of multiple variants. It is also possible that both mechanisms may occur within a host at different time points and anatomical locations of infection.

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

The address of the proposed site of release is: Oxford Vaccine Group, Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), Churchill Hospital, Old Road, Headington, Oxford OX3 7LE. The national (OS) grid reference for the proposed site release is SP543060.

As a consequence of shedding through faecal material the GMO may be released into the sewage system in England and primarily within the Oxfordshire area. The sewage system is designed to contain and clear bacteria including *Salmonell*a.

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).

In this clinical research study we propose to utilise the human challenge model established at the Oxford Vaccine Group (UK) to investigate the nature of the bacteraemia that results when a 1:1 mixture of two *Salmonella* Typhi strains are administered at the same time. The primary outcome measure will be to determine the proportion of participants who develop bacteraemia post challenge with wild type *Salmonella* Typhi Quailes strain (WT) or a typhoid toxin-deficient isogenic mutant of *Salmonella* Typhi Quailes strain SB6000 (GMO) or a combination of both strains. This information will be used to investigate the 'bottleneck' hypothesis, the idea that it might be only one bacteria that crosses the gut lining to cause a bloodstream infection. The results of this study may also inform vaccine design and development potentially influencing public health intervention strategies. The title of the project is 'Exploring the bottleneck hypothesis of the pathogenesis of bacteraemia in an ambulatory outpatient human experimental infection of *Salmonella* Typhi'.

B5 The intended dates of the release.

The exact timing of the release will be dependent on all study approvals being in place. We anticipate that clinical study will commence on the 1st of March 2019 (pending all necessary approvals). A minimum of 6 participants and a maximum of 15 participants will be enrolled to the study from within the Oxfordshire area. The GMO will be given orally to study participants who are likely to shed the organism in faeces/stool. This shedding constitutes the release of the GMO. We anticipate the trial will run for at least 18 months but this may be longer if there are difficulties with recruitment or if follow on studies involving the release of this GMO are required hence release may occur until March 2023.

B6 The environmental risk assessment.

The GMO is an isogenic mutant of the wild type (parent) *S*. Typhi Quailes strain and has been designed and manufactured to GMP standard for use as an oral challenge agent within a controlled human infection model to be conducted at the Oxford Vaccine Group (University of Oxford, UK). The GMO will be administered orally to healthy volunteers recruited to the study who are likely to shed the organism in faeces/stool. This will constitute the release of the organism resulting in release into the sewage system. Normal basic hygiene precautions, namely the use of toilets and hand washing, are considered sufficient to prevent person to person transmission. The GMO is expected to be effectively contained and inactivated by usual sewage system processes.

Wild type *S*. Typhi has no known animal reservoir (human restrict pathogen) and the GMO will not have a selective or survival advantage. The potential for genetic exchange with any other organism in the environment is extremely low given that the GMO does not contain any plasmids or antibiotic resistance markers. The GMO is not expected to persist in study participants following the completion of 7 days of antibiotic treatment.

Survival and Persistence Characteristics
To evaluate the survival and persistence characteristics of both the wild type parent strain (Quailes) and typhoid toxin-deficient isogenic mutant (SB6000) a series of experiments were conducted in river water, sea water and soil. In conclusion the data demonstrate that the GMO strain SB6000 did not persist for any longer than the wild type Quailes strain from which it is derived from. The data demonstrate that both strains survive only transiently in the environment. The wild type and SB6000 strains are phenotypically similar with respect to persistence.

River and sea water:
River water aliquots were inoculated with the *S*. Typhi wild type (WT) and SB6000 GMO strains. All aliquots were then incubated statically at 21°C and analysed at various time points following inoculation. At each time point the viable cell count (VCC) of the samples was determined and the study proceeded for each sample until no viable cells were recovered in 1ml of water. The rate of decline over a period of 68 days was similar for both the WT and GMO (SB6000) strains (Figure 1).

Sea water aliquots were then incubated statically at 21 °C and analysed at various time points following inoculation. At each time point the VCC of the samples was determined and the study proceeded for each sample until no viable cells were recovered in 1 ml of water. Neither the GMO (SB6000) nor the WT *S*. Typhi persisted beyond day 51 (Figure 1).

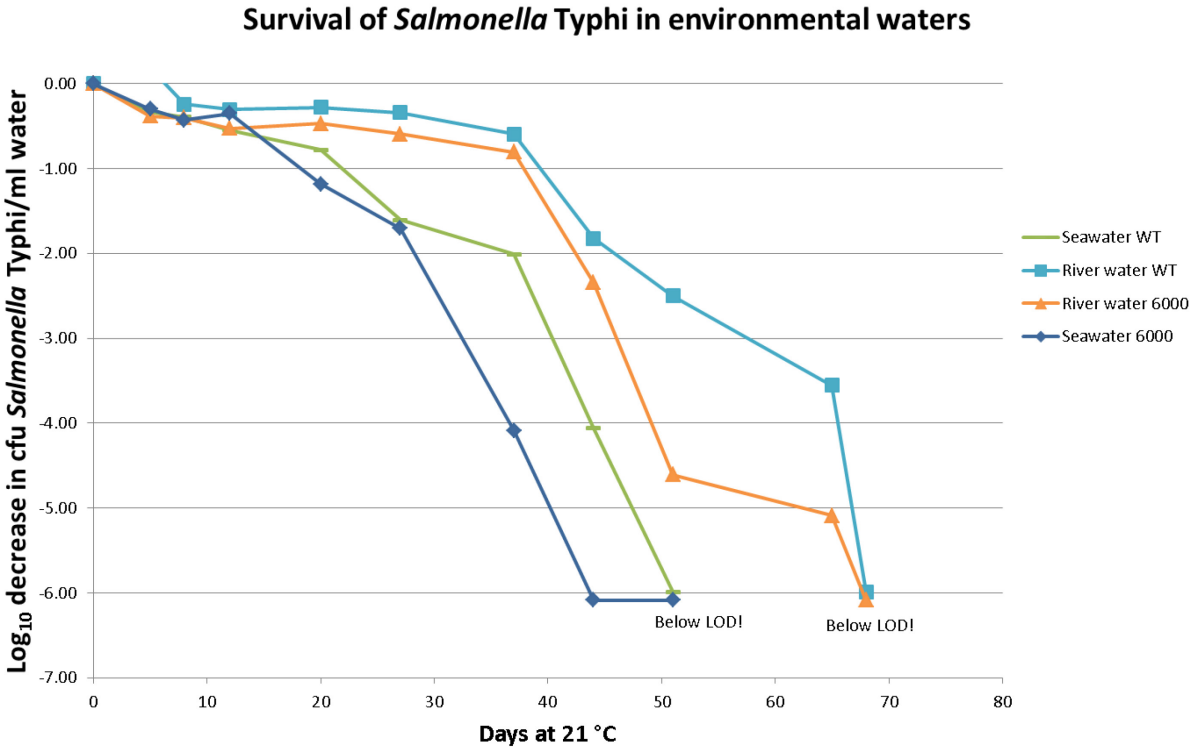

Figure 1. Survival of survival of *Salmonella* Typhi WT strain Quailes and its SB6000 GMO derivative in river and sea water.

Soil:
Aliquots of top soil were inoculated with the *S*. Typhi WT or SB6000 GMO strains. All aliquots were then incubated statically at 21 °C and analysed at various time points following inoculation. At each time point the viable cell count of the samples was determined and the study proceeded for each sample until no viable cells were recovered in 1 ml of soil suspension. It was concluded from this study that the GMO survived for only a limited time in soil. The GMO persisted for no longer than 44 days at the highest inoculum level tested and no longer than 37 days at the lower inoculum level (Figure 2).

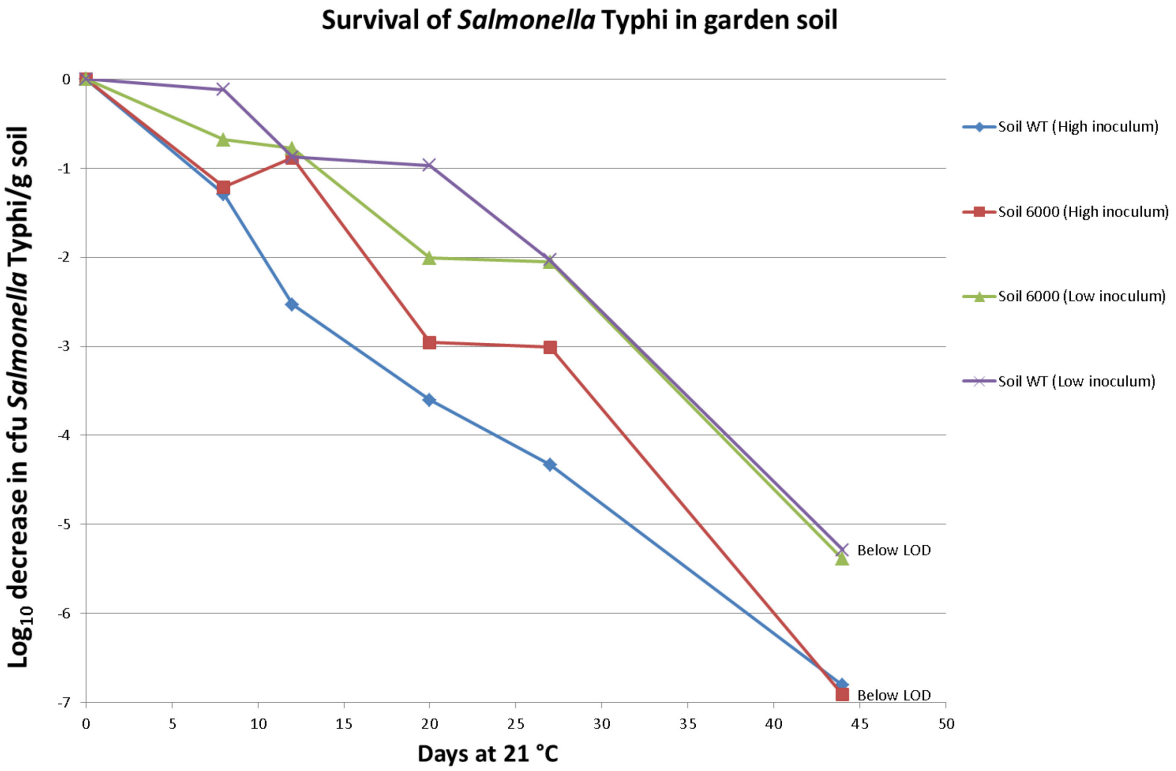

Figure 2. Survival of *Salmonella* Typhi WT strain Quailes and its SB6000 GMO derivative in garden soil.

For the purpose of the release, the GMO will be given to a minimum of 6 participants and a maximum of 15 participants at a single site in Oxford. Additional participants may receive the GMO to account for participant withdrawals before the completion of the antibiotic treatment period of the study. The study participants will be healthy adult volunteers aged 18-60 years. During the initial post-challenge phase the study participants must be resident within Oxfordshire (up to 28 days post challenge). Stool clearance will be confirmed following 3 negative stool samples provided at least 48 hours apart following completion of a 7 day course of antibiotics. The GMO has previously been administered to healthy subjects in a clinical study conducted at the Oxford Vaccine Group and the safety profile of the GMO was comparable to that of the wild-type strain. The risk of both the GMO and wild-type strain to other humans is considered to be low and the risk to the environment is considered to be effectively zero.

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

Methods and plans for monitoring the GMO:
The health and safety of our study participants is of the upmost importance and will be actively and closely monitored for the duration of the study. Any symptoms will be clinically managed by the site study physicians as appropriate.

Stool cultures will be taken at Day 0 (challenge), throughout the 14 day post-challenge period and at visits after typhoid diagnosis. Time to onset of stool shedding - time from challenge (Hours/Days) to the first positive stool culture, excluding the first 24 hours following ingestion of challenge agent will be documented. Participants will be required to supply further stool samples until proven not to be shedding *S.* Typhi. Stool samples will be collected at least one week after completion of a 7 day course of antibiotics, until three successive stool samples are negative for *S*. Typhi Three stool samples. If persistent stool shedding occurs after completion of antibiotics, participants will be referred to the Infectious Diseases Consultant at the Oxford University Hospitals NHS Foundation Trust. Additionally, quantitative stool cultures or PCR may be performed to assess the burden of stool shedding. Isolates from stool samples will be stored frozen for future analysis, which may include phage typing or genetic sequencing.

Frequency of the monitoring:
Monitoring will take place for the duration of the clinical study. The study is expected to commence on the 1st of March 2019 (pending all necessary approvals). All study participants will have follow-up visits up to 6 months post challenge. We anticipate the trial will run for at least 18 months but this may be longer if there are difficulties with recruitment or if follow on studies involving the release of this GMO are required hence release may occur until March 2023.

Following challenge with the GMO and wild type Quailes strain participants will be monitored daily for the first 14 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. The protocol for visits will depend on whether the participant develops infection or not. Following diagnosis of enteric fever blood and stool sampling will be performed at 6, 12, 24, 48, 72 and 96 hours post diagnosis. Following completion of antibiotic treatment and confirmed clearance of the GMO in stool samples participants will be monitored a long term follow-up visits at Day 28, 90 and 180. All study participants will agree to have 24-hour contact with study staff during the four weeks post challenge and to be able to ensure that they are contactable by mobile phone for the duration of the challenge period until antibiotic completion.

Blood samples will be monitored daily for *S*. Typhi using a combination of microbiological and molecular biology techniques. The Oxford Vaccine Group has developed a fast and highly sensitive novel TSB-bile blood culture-PCR assay which has been used to detect low levels of *S.* Typhi in the blood of participants after challenge. A continuous monitoring system will be used to culture GMO organisms which will be identified as *S*. Typhi via biochemical and serological methods. Confirmed isolates will be tested for antibiotic susceptibility using standard microbiological methods.

Participants will be screened for shedding of *S*. Typhi in the stool. Stool cultures will be taken at Day 0 (challenge), throughout the 14 day post-challenge period and at visits after typhoid diagnosis. Participants will be required to supply 3 further stool samples until proven not to be shedding *S.* Typhi. To detect chronic carriage of *S*. Typhi, stool samples for culture will be obtained one week after completion of the antibiotic course until three samples (each taken at least 48 hours apart) are negative. Once these criteria are satisfied, the participant will be

considered to be fully treated for *S.* Typhi infection and no longer an infection risk. If samples remain positive for *S.* Typhi four weeks after completion of antibiotics then the participant will be referred to a Consultant in Infectious Diseases (Oxford University Hospitals NHS Foundation Trust) for further management. No evidence of stool shedding of the wild type *S.* Typhi Quailes strain after treatment or transmission to secondary contacts has been detected in previous challenge studies conducted at the Oxford Vaccine Group.

The Thames Valley Health Protection Unit (Public Health England) will be informed of all participants who have been challenged with *S.* Typhi, satisfy the definition of typhoid infection, have commenced and completed antibiotics, and have completed clearance stool sampling (with additional information and continued contact if persistence stool shedding occurs). The participants GP will also be notified at the time of stool shedding clearance. In addition any breaches in enteric precautions that result in another individual coming into contact with the excreta of a participant will be reported to Public Health England.

Stringent precautions are in place to avoid the spread of the GMO from the study participant to others. Such spread has not been noted in previous studies conducted by the Oxford Vaccine Group using the wild type *S.* Typhi Quailes strain. Person to person transmission will be prevented by normal basic hygiene practice (primarily the use of toilets and hand washing). In view of the low infectivity of *S.* Typhi (without administration in sodium bicarbonate buffer) and the high standard of hygiene and sanitation in the UK, secondary transmission of the challenge strain to household or other close contacts is highly unlikely. It is acknowledged, however, that transmission within households can occur if the individual excreting *S.* Typhi fails to practice effective hand washing after defecation and is subsequently involved in uncooked food preparation. The participant will provide letters from the study team to close contacts including household contacts. Contacts will be offered the opportunity to be screened for *S.* Typhi infection, which will involve obtaining two stool samples 48-hours apart. If either stool culture of a household contact is positive, he/she will be referred to a Consultant in Infectious Diseases for appropriate antibiotic management and Public Health England will be informed.

Methods and plans for responding to an emergency:
Participants who vomit for any reason within 90 minutes of the challenge will be withdrawn from the trial and treated with antibiotics. This will be treated as an emergency spill of the GMO and standard operating procedures will be followed by the research team. Suitable personal protective equipment and disinfectants will be used to inactive the GMO. All waste will be autoclaved prior to disposal from the site according to local GMO and University of Oxford standard operating procedures.

Participants 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 four weeks post challenge and to be able to ensure that they 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. In addition, participants agree to allow the study team to hold the name and 24-hour contact number of a close friend, relative or housemate who will be kept informed of the study participant's whereabouts for the duration of the challenge period (from the time of challenge until completion of antibiotic course). This person will be contacted if study staff are unable to contact the participant.

Participants will be issued with a Medic Alert-type card containing information including the antibiotic sensitivity of the *S.* Typhi strain (GMO and wild type Quailes strain), 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 and second line antibiotics (ciprofloxacin, azithromycin or other macrolide antibiotics) will be excluded from the study. The antibiotics to be used in this study are generally well tolerated and are only occasionally associated with side effects. Should an antibiotic cause allergy or intolerance this will be managed by a study doctor and a different antibiotic will be used for subsequent management. The participant's GP will be notified in writing of the antibiotics received. Participants will receive telephone calls or text messages to remind them to take their antibiotic dose.

There are provisions within the protocol and site facilities to allow for admissions of participants as inpatients to the John Warin Ward (John Radcliffe Hospital, Oxford) in cases of severe typhoid fever and/or other circumstances.