A human controlled infection study to assess colonisation and immunogenicity following nasal inoculation with *Neisseria lactamica* with eradication on Day 4 or 14

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Chief investigator: Professor R.C. Read

University Hospital Southampton NHS Foundation Trust

Version	Date	Authors	Modifications
1.0	18th July 2016	Diane Gbesemete Hans de Graaf Robert Read	
2.0	26th September 2016	Diane Gbesemete Hans de Graaf Robert Read	Increase in sample size by 10% to allow for participant withdrawal Additional follow up visits on day 32 for group 1 and days 7 and 42 for group 2 Correction of current inoculum details in section 6.2 and 6.3 Correction of SOP titles throughout document Alteration of exclusion criteria - Addition of current colonisation with *N. meningitidis* - Alteration in exclusion duration following involvement in other clinical trials Alteration in safety requirements - No negative pressure room required for inoculation - No requirement for staff logging - Alteration of staff presence requirement on unit to comply with current unit guidelines Study procedures - Addition of saliva samples at 3 time points - Blood tests – addition of cellular immunology - Increase in total blood volume to 60ml - Additional throat swab at each time point - Repeat of all investigations at 28 days post eradication - Further minor changes in schedule of procedures - Additional pregnancy test prior to eradication therapy Alteration of study endpoints to correspond to altered study procedures Addition of newsletter for dissemination of study results to volunteers Further changes in wording throughout the protocol following discussions among the research group
3.0	13th December 2016	Diane Gbesemete Hans de Graaf Robert Read	Alteration of definition of start / end of trial to include screening and data analysis Addition of environmental screening tests at follow up visits Minor changes / corrections in wording throughout protocol

4.0	1st March 2017	Diane Gbesemete Hans de Graaf Robert Read	Clarification of exclusion criteria with the addition of an ECG Modification of study procedures tables – addition of windows to study visits, ECG and footnotes to clarify optional samples
5.0	26th April 2017	Diane Gbesemete Hans de Graaf Robert Read	Alteration of exclusion criteria to include nasal wash culture positive for *N. lactamica* or *N. meningitidis*

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Study reference: RHM MED1354

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Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the sponsor, the investigator team, and members of the independent ethics committee. This information cannot be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Professor R.C. Read**.**

Investigator Agreement

"I have read this protocol and agree to abide by all provisions set forth therein.

I agree to comply with the principles of the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice."

Professor R.C. Read	……	……
Chief Investigator	Investigator Signature	Date

Conflict of Interest

1. “According to the Declaration of Helsinki, 2008, I have read this protocol, and declare no/the following (delete as appropriate) conflict of interest”

Professor R.C. Read	……	……..
Chief Investigator	Investigator Signature	Date

2. “According to the Declaration of Helsinki, 2008, I have read this protocol, and declare no/the following (delete as appropriate) conflict of interest”

Professor S.N. Faust	……	……..
Principal Investigator	Investigator Signature	Date

3. “According to the Declaration of Helsinki, 2008, I have read this protocol, and declare no/the following (delete as appropriate) conflict of interest”

D. Gbesemete	……	……..
Investigator	Signature	Date

4. “According to the Declaration of Helsinki, 2008, I have read this protocol, and declare no/the following (delete as appropriate) conflict of interest”

H. de Graaf	……	……..
Investigator	Signature	Date

Table of Contents

1. Synopsis

Title	**A human controlled infection study to assess colonisation and immunogenicity following nasal inoculation with *Neisseria lactamica* with eradication on Day 4 or 14**
Sponsor	University of Southampton NHS Foundation Trust
Trial Centre	NIHR Wellcome Trust Clinical Research Facility, Southampton University Hospital NHS Foundation Trust, Southampton, SO16 6YD
Trial Sponsor Code	RHM MED1354
Design	Phase II prospective controlled human *Neisseria lactamica* infection study
Population	Healthy volunteers aged 18-45 years
Sample size	Total up to 44 volunteers Group 1: Nasal inoculation with *Neisseria lactamica* with eradication on day 4 – up to 22 volunteers or until 11 colonised on day 4 Group 2: Nasal inoculation with *Neisseria lactamica* with eradication on day 14 – up to 22 volunteers or until 11 colonised on day 4
Follow up duration	Group 1: Challenge at day 0, eradication on day 4, follow up on days 5, 14 and 32 Group 2: Challenge at day 0, follow up on day 4 and 7, eradication on day 14, follow up on day 15, 24 and 42
Planned Trial Period	13/03/2017– 30/9/2018
Primary Objective	To compare the effect of short (4 days) versus longer (14 days) oropharyngeal carriage on systemic and mucosal humoral immunogenicity following nasal inoculation with *Neisseria lactamica*
Secondary Objective	To assess the efficacy of oral Ciprofloxacin in eradicating nasal *Neisseria lactamica* colonisation by 24 hours after treatment
Microbial challenge material	*Neisseria lactamica* Wild type – dose 100000 (10^5) cfu

2. Abbreviations

AE	Adverse Event
ALP	Alkaline Phosphatase
ALS	Advanced Life Support
ALT	Alanine transaminase
AR	Adverse Reaction
cfu	Colony Forming Unit
CRF	Case Report File
DSUR	Development Safety Update Report
ESC	External Safety Committee
GCP	Good Clinical Practice
GP	General Practitioner
HRA	Health Research Authority
ICH	International Conference on Harmonisation
ILS	Immediate Life Support
MHRA	Medicine and Healthcare products Regulatory Agency
NHS	National Health Service
NIHR	National Institute for Health Research
NIHR-WTCRF	NIHR Wellcome Trust Clinical Research Facility
Nlac	*Neisseria lactamica*
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
QA	Quality assurance
RBC/HPF:	Red blood cell per high power field
REC	Research Ethics Committee
SAE	Serious Adverse Event

SAR	Serious Adverse Reaction
SmPC	Summaries of Product Characteristics
SOP	Standard Operating Procedure
SUSAR	Serious Unexpected Serious Adverse reaction
TOPS	The Over-volunteering Prevention System
UAR	Unexpected Adverse Reaction
UHS NHS FT	University Hospital Southampton NHS Foundation Trust

3. Background and rationale

3.1 Vaccines and immunity to carriage

Childhood glycoconjugate vaccines (e.g. meningococcal, pneumococcal and *Haemophilus*) have been shown to be highly effective due to both their direct effect on the immunity of the individual and also as a result of herd protection. This herd protection is a result of vaccine-induced modification of colonisation reducing inter-host transmission. Future successful vaccines will target pathogen colonisation, using antigens known to induce immunity critical for colonisation, in age groups most likely to transmit to others. The most direct and effective way to measure carriage reduction by vaccines is by inducing carriage of a defined organism expressing antigens of interest (experimental human challenge) in persons exposed to vaccine and measuring the subsequent carriage of the bacterium.

3.2 Immunity in natural carriage

Colonisation by bacteria is an immunising event; we proved this in humans by inoculating university students intranasally with the harmless commensal *N. lactamica* and we observed both specific systemic and mucosal antibody responses by 4 weeks (Evans, Pratt et al. 2011). Likewise a human challenge model has been used to study responses to pneumococci in which, in addition to humoral responses, T cell responses were induced by asymptomatic carriage of wild-type *S. pneumoniae,* with an increase in IL-17A$_+$ Th cells in both the blood and bronchoalveolar lavage fluid by 21-56 days post challenge, suggesting that similar to animal models of carriage, Th17-mediated responses are also mobilised in humans (Wright, Bangert et al. 2013). Assessment of the breadth of cellular responses to bacterial carriage suggests that unlike humoral responses, they are not restricted to the most immunodominant antigens (Davenport, Guthrie et al. 2003) (Glennie, Banda et al. 2012). However, repeated colonisation by the same species is known to occur throughout the lifetime of humans (Bidmos, Neal et al. 2011). Experimental challenge with defined bacteria could tease out the mechanisms of this, which include waning of immunity over time, the induction of an incorrectly polarised T cell response, lack of cross-reactivity between strains or active immune evasion mechanisms employed by bacteria to subvert host immune effector mechanisms.

3.3 *Neisseria lactamica*

Neisseria lactamica is a non-pathogenic lactose-fermenting Gram-negative diplococcus, frequently found in the nasopharynx, particularly in young children. Transmission occurs through close contact and only a few cases of clinical significance have been reported (Denning and Gill 1991) (Brown, Ragge et al. 1987) (Lauer and Fisher 1976). Although *N. lactamica* and *N. meningitidis*, colonise the same location within the upper respiratory tract, previous studies suggest they engage with the human mucosal immune system in very different ways (Vaughan, Gorringe et al. 2009). In contrast to *N. meningitidis*, *N. lactamica* maintains a commensal relationship with the host in the absence of an adaptive immune response. *N. lactamica* lacks a polysaccharide capsule, so any adaptive immune responses to this bacterium must be directed at non-capsular antigens, providing a good platform for assessing non-anti-polysaccharide immunity against colonising bacteria when compared with wild type *N. lactamica*.

3.4 *Neisseria lactamica* for human challenge

N. lactamica (Nlac) has been shown to be safe in human challenge as we have found in over 340 volunteers experimentally inoculated with this organism (Evans, Pratt et al. 2011) (Deasy, Guccione et al. 2015). Even at a very low dose of 10^4 colony forming units (cfu), long lasting colonisation with Nlac is easily induced in 35-65% of participants. We showed (Evans, Pratt 2011) that increasing the inoculum to 10^5 cfu increased the subsequent carriage of *N. lactamica* to 50%. In 80-90% of those successfully colonised, this is detectable by 1-2 weeks. Data regarding earlier detection of colonisation is currently lacking. (Evans, Pratt et al. 2011) (Deasy, Guccione et al. 2015) Colonisation has a clear effect on the nasal mucosal microbiome, in that meningococcal acquisition is effectively inhibited in participants who carry the organism (Deasy, Guccione et al. 2015). Colonisation is immunogenic with an increase in specific serum IgG by 2 weeks and specific salivary IgA by 4 weeks (Evans, Pratt et al. 2011). No eradication therapy has previously been given following experimental inoculation but Ciprofloxacin has been shown to be effective in the eradication of *N. meningitidis* with clearance in 100% of previously colonised individuals after 24 hours. (Pugsley, Dworzack et al. 1987) To design future planned studies using *N. lactamica* nasal inoculation and colonisation in order to prevent invasive *N. meningitidis* disease, it is necessary to further evaluate the colonisation kinetics and efficacy of eradication following antibiotic treatment. Planned studies include infection with *N. lactamica* engineered to express antigens included in modern vaccines such as NadA and PorA. Such studies will require containment of volunteers and for ethical and economic reasons it will be necessary to make the period of colonisation as short as possible, and to eradicate carriage of the organism at the end of the experiment. Previous challenges we have done with *N. lactamica* have allowed the volunteers for prolonged periods of follow-up over 6 months.

3.5 General overview

This study will compare the effect of short (4 days) versus longer (14 days) periods of nasal carriage of *N. lactamica* on immunogenicity and confirm the efficacy of oral eradication therapy with ciprofloxacin. Healthy adult volunteers will receive a nasal inoculation of *N. lactamica* and eradication therapy will be given on day 4 or 14. This information will be used to inform the design of future research into the colonisation kinetics and immunogenicity of related organisms. Specifically, the Medical Research Council has funded us to inoculate humans with genetically modified *N.lactamica*. Before designing the protocols for that study we need to know whether a short containment of the volunteers will be sufficient for immunogenicity, and how quickly we can discharge the volunteers after ciprofloxacin eradication. A wild-type strain of *N. lactamica* Y92-100 will be used for this study, selected because we have previously used it safely in experimental challenge of over 340 human volunteers. The same strain is the parent strain for the GMO work described above.

4. Objectives

4.1 Primary objective

- To compare the effect of short (4 days) versus longer (14 days) oropharyngeal carriage on systemic and mucosal humoral immunogenicity following nasal inoculation with *Neisseria lactamica*

4.2 Secondary objectives

- To assess the efficacy of oral ciprofloxacin in eradicating nasal *Neisseria lactamica* colonisation by 24 hours after treatment

5. Description and justification of the study design

5.1 Overview

This is a prospective controlled human challenge study in which participants will be inoculated intranasally with *N. lactamica* and eradication therapy will be given at day 4 or day 14. Colonisation will be assessed at day 4 for both groups and day 7 and 14 for group 2. Efficacy of eradication will be checked 1 day and 10 days after eradication therapy is given (day 5 and day 14 for group 1, day 15 and day 24 for group 2) and immunogenicity will be assessed at day 14 post inoculation and day 28 post eradication.

5.2 Study volunteers

Healthy volunteers aged 18-45 years will be recruited and challenged until 11 volunteers in each group are colonised with *N. lactamica* at day 4 or up to a maximum of 44 volunteers in total. Based on our previous studies with *N. lactamica,* colonisation was achieved in 35% of *N. lactamica* -inoculated non-smokers at a dose of 10^4 cfu, (Deasy, Guccione et al. 2015) and increasing the inoculum to 10^5 cfu increased the subsequent carriage of *N. lactamica* to 50% (Evans, Pratt 2011). Therefore inoculating up to 44 participants will result in approximately 22 colonised individuals.

5.3 Randomisation and blinding

There will be no randomisation or blinding in this study. Prior to enrolment volunteers can give their preference for early eradication at day 4, or late eradication at day 14. Once one group has reached 22 volunteers, any remaining volunteers will be recruited in the other group to ensure both arms include a maximum of 22 participants.

5.4 Challenge procedure

Our priority is to conduct this study without causing harm to the volunteer. As *N. lactamica* is a non-virulent commensal organism and based on previous challenge studies with this organism, we consider that the likelihood of disease resulting from inoculation of the volunteers is extremely low.

5.5 Duration of volunteer participation

The duration of involvement of volunteers in the study will be 32 days (group 1) or 42 days (group 2) from enrolment.

5.6 Definition of the start and the end of the trial

The start of the trial is defined as the date of screening of the first volunteer. The end of the trial is defined as 12 months after the date of the last visit of the last volunteer to allow for sample processing and data analysis.

5.7 Potential benefits for the volunteers.

Volunteers will not benefit directly from participation in this study. However, it is hoped that the information gained from this study will contribute to knowledge about nasal colonisation

and therefore to the development of a safe and effective nasal vaccine in the future. Volunteers will also receive information about their general health status.

5.8 Involvement of the NHS Research Ethics Committee

This study protocol has been submitted to Hampshire A Regional Ethics Committee (REC) for approval of this human challenge study.

6. Inoculum

6.1 Selection of strain of *N. lactamica*

A wild-type strain of *Neisseria lactamica* strain Y92-1009 (sequence type 3493, clonal complex 613) will be used because we have previously used it safely in experimental challenge of over 250 human volunteers (Deasy, Guccione et al. 2015, Evans, Pratt et al. 2011).

6.2. Production of the inoculum

Stocks of *N. lactamica* Y92-1009 (sequence type 3493, clonal complex [CC] 613) in Frantz medium containing 30% (v/v) glycerol will be supplied by the Current Good Manufacturing Practices pharmaceutical manufacturing facilities at Public Health England (Porton Down, United Kingdom). Vials of $1x10^6$ bacteria, suspended in Frantz medium containing 30% (v/v) glycerol, will be stored at -80°C and will be thawed and diluted in phosphate buffered saline (PBS) prior to inoculation, following the study specific SOP: **Preparation and monitoring of *N. lactamica* inoculum for human challenge studies**.

6.3 Quality assessment of the inoculum

Frozen stocks will be assessed for viability and contamination using standard microbiological procedures. Using these data a single batch will be prepared for storage at -80°C and subsequent human challenge. Stocks will be checked monthly for changes in viable counts.

6.4 Transport of the inoculum

The inoculum will be transferred to the University Hospital Southampton under temperature-monitored conditions. The aliquot will be placed into a container as secondary packaging. They will be placed inside leak and shock resistant transport boxes with secured lids.

6.5 Storage of the inoculum

The cell banks will be stored at -80°C in a locked, dedicated, temperature monitored freezer.

6.6 The optimal and safe dose of the inoculum

Based on the previous *N. lactamica* work it is estimated that 50% of volunteers will be colonised 1-2 weeks after inoculation with 10^5 cfu without any safety concerns (Deasy, Guccione et al. 2015). The hypothesis is that those who are colonised at day 4 will exhibit seroconversion by day 14, confirming biologically `significant` colonisation.

6.7 Monitoring of the *N. lactamica* dose administered to volunteers

After the inoculum is given a sample of the residual inoculum will be diluted and cultured overnight. The dose will then be evaluated by viable count and the species confirmed by Polymerase Chain Reaction (PCR).

7. Recruitment and withdrawal of trial volunteers

7.1 Recruitment

Healthy volunteers will be recruited through various media. Care will be taken not to recruit from vulnerable groups (mental health or other capacity issues or those under 18 years old). The recruitment strategy will be approved by the Health Research Authority (HRA). Volunteers may be recruited by use of an advertisement +/- registration form formally approved by the ethics committee and distributed or posted in the following places:

- In public places, including buses and trains, with the agreement of the owner / proprietor
- In newspapers or other literature for circulation
- On radio via announcements
- On a website operated by our group or with the agreement of the owner or operator (including on-line recruitment through our web-site)
- As a post on a Twitter, Facebook or Gumtree account owned and operated by our group
- Video message posted on the National Health Service (NHS) YouTube channel.
- By e-mail distribution to a group or list only with the express agreement of the network administrator or with equivalent authorisation
- By email distribution to individuals who have already expressed an interest in taking part in any clinical trial at the NIHR-WTCRF Southampton
- On stalls or stands at exhibitions or fairs
- Via presentations (e.g. presentations at lectures or invited seminars)
- Direct mail-out: This will involve obtaining names and addresses of adults via the most recent Electoral Roll. The contact details of individuals who have indicated that they do not wish to receive postal mail-shots would be removed prior to the investigators being given this information. The company providing this service is registered under the Data Protection Act 1998. Investigators would not be given dates of birth or ages of individuals but the list supplied would only contain names of those aged between 18-45 years (as per the inclusion criteria)
- Southampton NIHR-WTCRF Database of Healthy Volunteers: We may contact individuals from this database who have previously expressed an interest in receiving information about future studies for which they may be eligible

7.2 Volunteer information sheet

A volunteer information sheet will be given to the volunteer at least 24 hours before the screening visit. The volunteer information sheet will include all risks of participating in this study and safety measures that are involved in the study and will be formally approved as part of the HRA application.

7.3 Screening visit

Individuals who have expressed an interest in taking part in the study will be invited to attend a screening session after a short telephone screening. During the screening visit the study will be explained. If the volunteer has any questions they can be addressed during this visit. The screening visit can be up to 90 days prior to challenge on day 0.

7.3.1 Informed consent

All volunteers will sign and date the informed consent form before any study specific procedures are performed. At the screening visit, the volunteer will be fully informed of all aspects of the trial, the potential risks and their obligations. The following will be emphasised:

- Participation in the study is entirely voluntary
- Refusal to participate involves no penalty or loss of medical benefits
- The volunteer may withdraw from the study at any time
- The volunteer is free to ask questions at any time to allow him or her to understand the purpose of the study and the procedures involved
- There is no direct benefit from participating
- The volunteer will be registered on the TOPS database (The Over-volunteering Prevention System; www.tops.org.uk)
- The aims of the study and all tests to be carried out will be explained. The volunteer will be given the opportunity to ask about details of the trial, and will then have time to consider whether or not to participate

The volunteer will be asked to sign and date two copies of the consent form, which will also be signed and dated by the investigator. One original will be given to the volunteer and the other will be stored in the NIHR-WTCRF. A copy will be stored in the volunteer's medical file.

7.3.2 Medical history and physical examination

A detailed medical history and physical examination will be conducted, making sure that all inclusion criteria and no exclusion criteria are met.

7.3.3 Screening investigations

A throat swab will be taken to look for *N. lactamica* and *N. meningitidis* carriage at screening. Females will have a pregnancy test. A nasal wash will be taken according to the SOP: **Collection of a nasal wash sample** for culture and as a baseline for assessing mucosal immunogenicity. An ECG will be performed at screening or prior to inoculation.

7.4 Inclusion and exclusion criteria

7.4.1 Inclusion criteria

The volunteer must satisfy all the following inclusion criteria to be eligible for the study:

- Healthy adults aged 18 to 45 years inclusive on the day of enrolment
- Fully conversant in the English language
- Able and willing (in the investigator's opinion) to comply with all study requirements
- Written informed consent to participate in the trial
- Willingness to take an antibiotic regimen after inoculation according to the study protocol
- For females only, willingness to practice continuous effective contraception (see below) during the study and a negative pregnancy test on the day(s) of screening and inoculation

7.4.1 Exclusion criteria

The volunteer may not enter the study if any of the following criteria apply:

- Current active smokers
- *N. lactamica* or *N. meningitidis* detected on throat swab or nasal wash taken before the challenge
- Individuals who have a current infection at the time of inoculation
- Individuals who have been involved in other clinical trials involving receipt of an investigational product over the last 12 weeks or if there is planned use of an investigational product during the study period
- Individuals who have previously been involved in clinical trials investigating meningococcal vaccines or experimental challenge with *N. lactamica*
- Use of systemic antibiotics within the period 30 days prior to the challenge
- Any confirmed or suspected immunosuppressive or immune-deficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) immunosuppressant medication within the past 6 months (topical steroids are allowed)
- Use of immunoglobulins or blood products within 3 months prior to enrolment.
- History of allergic disease or reactions likely to be exacerbated by any component of the inoculum
- Contraindications to the use of ciprofloxacin, specifically a history of epilepsy, prolonged QT interval, hypersensitivity to quinolones or a history of tendon disorders related to quinolone use
- Any clinically significant abnormal finding on clinical examination
- Any other significant disease, disorder, or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data, for example recent surgery to the nasopharynx
- Occupational, household or intimate contact with immunosuppressed persons
- Pregnancy or lactation

7.4.2 Effective contraception for female volunteers

Female volunteers are required to use an effective form of contraception during this study. Acceptable forms of contraception include:

- Established use of oral, injected or implanted hormonal methods of contraception
- Placement of an intrauterine device or intrauterine system
- Total abdominal hysterectomy
- Barrier methods of contraception (condom or occlusive cap with spermicide)
- Male sterilisation if the vasectomised partner is the sole partner for the subject
- True abstinence when this is in line with the preferred and usual lifestyle of the subject

8. *N. lactamica* challenge (day 0)

8.1 Trial site

The challenge will take place in the NIHR Welcome Trust Clinical Research Facility at University Hospital Southampton NHS Foundation Trust (UHS NHS FT). (http://www.uhs.nhs.uk/ClinicalResearchinSouthampton/Trials-and-facilities/NIHRWellcome-Trust-Clinical-Research-Facility/Our-facility.aspx).

8.2 Clinical team involved in the challenge

The challenge will be conducted by the study doctor together with a study nurse. The study doctor will be responsible for the administration of the inoculum. Advanced Life Support (ALS) trained doctors and Immediate Life Support (ILS) trained nurses will be present at inoculation and available within the NIHR-WTCRF for the post inoculation period of observation. At least 2 healthcare professionals will be available within the NIHR-WTCRF and a research clinician will be contactable by telephone whenever volunteers are present on the unit for follow up visits. The NIHR-WTCRF is situated in the University Hospital Southampton NHS Foundation Trust and a resuscitation team and intensive care facilities are available. Volunteers will have a 24 hours 7 days per week contact number to contact the Principal Investigator (PI) and research team in case of any adverse reactions during the study.

8.3 Infection control

The research team will adhere to the University Hospital Southampton Standard Infection Prevention and Control Precautions (Version 5 25/4/2016) for all patient contact and procedures including the challenge procedure.

8.4 Challenge Procedures

8.4.1 Confirmation of identity of the volunteer

Before any procedure is performed the identity of the volunteer will be confirmed by asking his/her name and date of birth and comparing it to the case report form and the label on the inoculum.

8.4.2 Review prior to the challenge

Prior to any procedure taking place the volunteer will be asked if he has any questions about the study and agrees to continue with the study. Eligibility will be reconfirmed before the challenge is conducted.

Medical history

The study doctor will take an interim medical history asking if there are any new medical issues since the screening visit. Special attention will be given to any possible signs of infection such as fever. If there are any significant abnormalities found, the challenge will be postponed at the discretion of the study doctor.

Physical examination

A physical examination will be conducted to check for any abnormalities. Vital signs including heart rate, blood pressure, respiratory rate and temperature will be recorded prior to the challenge procedure. If there are any significant abnormalities found, the challenge may be postponed at the discretion of the study doctor.

Laboratory investigations

A nasosorption test will be performed according to the SOP: **Collection of a nasosorption sample** to collect nasal fluid for assessment of mucosal immune responses. Two posterior pharyngeal swabs will then be taken according to SOP: **Collection of throat and nasal swabs (excluding samples for PCD) SCBR/GEN/270**. Saliva samples will be collected according to SOP: **Collection of saliva using an Oracol saliva swab**. Blood samples will be taken as a baseline for the assessment of cellular and humoral immunogenicity. Collection of all clinical samples will adhere to the UHS Standard Infection Control Precautions Policy (Version 5.0, 25/4/16). Assessment of shedding of *N. lactamica* will be carried out according to the study specific SOP: **Assessment of the environmental shedding of *N. lactamica***. This will involve analysis of a face mask worn for approximately an hour prior to study visits and / or speaking inside a cough box with air sampler.

8.4.3 Challenge

Members of the study team present at the challenge

The challenge will be conducted by the study doctor with a study nurse present.

Location of the challenge

The challenge will take place in the NIHR-WTCRF.

Time schedule of the challenge

The inoculation will take about 5 minutes, after which the volunteer will remain in the NIHR-WTCRF for further observation for a total of 30 minutes.

Preparing the inoculum

The inoculum will be prepared in the NIHR-WTCRF laboratory using a dedicated category II safety hood by technical staff. Two people will be present during preparation: one team member will prepare the inoculum, while the other team member will check the procedure which will be carried out according to the SOP: **Preparation and monitoring of *N. lactamica* inoculum for human challenge study**.

Administering the inoculum

The challenge procedure will be carried out by one of the study doctors according to study specific SOP: **Performance of nasal inoculation for human challenge studies**.

9 Clinical and laboratory monitoring

9.1 Clinical team

The clinical team involved in the monitoring of the volunteer will consist of the clinical investigators, NIHR-WTCRF research fellows and NIHR-WTCRF research nurses. At least two ILS or ALS trained staff members will be present on the NIHR-WTCRF throughout the period of observation following the challenge procedure. The NIHR-WTCRF is treated as a hospital ward so is covered by the hospital emergency response teams and medical intensive care support 24 hours per day.

9.2 Monitoring of volunteers

After the challenge the volunteers will be monitored in the NIHR-WTCRF for 30 minutes. They will have their vital signs measured at the end of this period of observation. A 24/7

emergency telephone contact number for the research team will be available in case of any concerns or systemic symptoms.

9.3 Potential adverse events

9.3.1 Phlebotomy

The maximum volume of blood drawn over the study period (60 mls) should not compromise these otherwise healthy volunteers. There may be minor bruising, local tenderness or pre-syncopal symptoms associated with venepuncture, which will not be documented as Adverse Events (AEs) if they occur.

9.3.2 Inoculation with N. lactamica

The inoculation with 0.5 millilitres of *N. lactamica* suspension can cause some irritation of the nasal mucosa that will disappear within a few seconds. Very occasionally, instillation may induce coughing or sneezing, but this can be prevented by slow instillation down the superior wall of the nares.

9.3.3 Nasosorption, throat swab, saliva sample and nasal wash

The nasal samples taken by nasosorption test, throat swab and nasal wash can cause some irritation of the nasal mucosa and can induce coughing or sneezing. This nasal discomfort will disappear within a few minutes and will not be recorded as an AE. The collection of saliva samples is not expected to cause any discomfort.

9.4.4 Environmental samples

The assessment of bacterial shedding by use of a mask or cough box may cause some distress to individuals with significant claustrophobia. If a volunteer feels they are unable to tolerate the face mask or cough box then they may miss out these study procedures but continue with the remainder of the study.

9.4 Withdrawal of volunteers

In accordance with the principles of the current revision of the Declaration of Helsinki (updated 2008) and any other applicable regulations, a volunteer has the right to withdraw from the study at any time and for any reason, and is not obliged to give his reasons for doing so. In addition the volunteer may withdraw/be withdrawn from further study procedures at any time in the interests of the volunteer's health and well-being, or for any of the following reasons:

- Administrative decision by the Investigator.
- Ineligibility (either arising during the study or retrospectively, having been overlooked at screening).
- Significant protocol deviation.
- Volunteer non-compliance with study requirements.
- An AE, which requires discontinuation of the study involvement or results in inability to continue to comply with study procedures.
- The reason for withdrawal from further study procedures will be recorded in the Case Report File (CRF). Except in case of complete consent withdrawal, long-term safety data collection will be continued. For all AEs, appropriate follow-up visits or medical care will be arranged, with the agreement of the volunteer, until the AE has resolved,

stabilised or a non-trial related causality has been assigned. Any volunteer who withdrew consent or is withdrawn from further study procedures may be replaced.

- If a volunteer withdraws from the study, blood samples collected before their withdrawal from the trial will be used/stored unless the volunteer specifically requests otherwise. Data from volunteers withdrawn from the study will be included in the analysis of results relating to the study's primary objective.
- In all cases of subject withdrawal, excepting those of complete consent withdrawal, long-term safety data collection will continue as appropriate if subjects have received the inoculum.

10. Follow up visits

Volunteers will attend for follow up visits as detailed in tables 10.1 and 10.2. At each visit the volunteers will be assessed for local and systemic adverse events and concomitant medication use. Measurement of vital signs and physical examination will be performed if clinically indicated and laboratory samples will be taken at the time-points indicated in the schedule of attendances. An appointment will be made for the next follow up visit.

Group 1	Screening	Inoculation and follow up			
Timeline (days)	≤ 90	0	4	5	14	32
TOPS confirmation	+					
Visit window			0	0	+/- 2	+/- 2
Volunteer Information Sheet	+					
Informed consent	+					
Vital signs	+	+	(+)	(+)	(+)	(+)
Medical history	+					
Physical examination	+	(+)	(+)	(+)	(+)	(+)
Pregnancy test (females only)	+	+	+			
Electrocardiogram	+	+[a]				
Review eligibility		+				
Inoculation		+				
Eradication			+			
Review of adverse events and concomitant medications			+	+	+	+
Nasal wash	+				+	+
Throat swab x 2	+[b]	+	+	+	+[b]	+[b]
Nasosorption test		+			+	+
Saliva sample		+			+	+
Environmental samples			+	+[c]	+[c]	+[c]
Immunological blood tests (ml)		20			20	20
Cumulative blood volume (ml)		20			40	60

Table 10.1: Summary of monitoring procedures during the study – Group 1; (+) if clinically indicated, [a]if screening ECG not available, [b]Throat swab 2 optional, [c]if colonised at previous visit

Group 2	Screening	Inoculation and follow up						
Timeline (days)	≤ 90	0	4	7	14	15	24	42
Visit window (days)			0	+/-2	+/-2	0*	+/-2	+/-2
TOPS confirmation	+							
Volunteer Information Sheet	+							
Informed consent	+							
Vital signs	+	+	(+)	(+)	(+)	(+)	(+)	(+)
Medical history	+							
Physical examination	+	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Pregnancy test (females only)	+	+			+			
Electrocardiogram	+	+[a]						
Review eligibility		+						
Inoculation		+						
Eradication					+			
Review of adverse events and concomitant medications			+	+	+	+	+	+
Nasal wash	+				+			+
Throat swab x 2	+[b]	+	+	+[b]	+	+	+[b]	+[b]
Nasosorption test		+			+			+
Saliva sample		+			+			+
Environmental samples			+	+[c]	+[c]	+[c]	+[c]	+[c]
Immunological blood tests (ml)		20			20			20
Cumulative blood volume (ml)		20			40			60

Table 10.2: Summary of monitoring procedures during the study – Group 2; (+) if clinically indicated, *1 day following D14 visit, [a]if screening ECG not available, [b]Throat swab 2 optional, [c]if colonised at previous visit, [c]1 day following D14 visit

10.1 Eradication therapy

Colonisation with *N. lactamica* will be eradicated by treatment with ciprofloxacin 500 mg single oral dose (BNF 2013, p350). Ciprofloxacin proved effective at eradicating *N. meningitidis* (Fraser, Gafter-Gvili et al. 2006*)* and should eradicate *N. lactamica* carriage within 24 hours (Pugsley, Dworzack et al. 1987). The strain used is fully sensitive to this antibiotic with an exceptionally low MIC on E-testing.

Eradication therapy will be given at day 4 for group 1 and day 14 for group 2. Eradication efficacy will be analysed by taking a throat swab 24 hours, 10 days and 28 days after eradication therapy (Day 5, 14 and Day 32 for Group 1, Day 15, 24 and Day 42 for Group 2).

Female volunteers will have a further pregnancy test prior to administering eradication therapy as pregnancy is a relative contraindication to its use. If a volunteer is found to be pregnant at this point they will not receive eradication therapy and will be withdrawn from the study.

10.1.1 Compliance with the eradication therapy

The dose will be taken under supervision of the study team (directly observed treatment)

10.1.2 Side effects of the eradication therapy

The side effects of ciprofloxacin in young adults may include:

- Abdominal ache, diarrhoea and nausea.
- Tiredness and headaches.
- Rash and itching.
- Facial swelling - very rarely breathing difficulties may occur with the facial swelling.
- Pain and inflammation around the joints

Volunteers should contact the clinical study team if this occurs.

Female volunteers using the oral contraceptive pill will be advised to use alternative forms of contraception for two weeks following eradication therapy as ciprofloxacin may interfere with the oral contraceptive pill.

10.2 Emergency contact

Subjects will be encouraged to contact one of the investigators on the 24 hour emergency mobile study telephone number if they develop symptoms between the regular follow up visits. The investigator will consider an extra clinical review if the volunteer has any symptoms that are moderate or severe.

11 Laboratory procedures

11.1 Laboratory work

Standard operating procedures for all laboratory work will be followed. Investigators will conform to well established laboratory safety standards.

11.2 Processing and storage of samples

Blood samples

Blood samples for immunological assays will be processed in the NIHR-WTCRF lab and frozen at -80ºC.

Immunological assays (serology and cellular immunology specific to *N. lactamica*) will be conducted according to the procedures established in the test laboratories.

Nasal washes, nasosorption test strips and saliva samples

Nasal wash fluid, nasosorption test strips and saliva samples will be processed in the NIHR-WTCRF lab and frozen at -80ºC and then analysed according to the procedures established in the test laboratories.

Throat swab 1 – bacterial culture and PCR

One throat swab at each time point will be placed into medium and centrifuged. The resulting solution will be spot plated and then incubated for up to 48hrs prior to the identification process. Positively identified Neisseria species will then be sub-cultured onto fresh plates for up to 24hrs. Individual colonies will be selected for storage in glycerol medium and frozen to

-80ºC to await PCR and sequencing for exact characterisation. The remainder of the solution will be frozen at -80ºC for a RT PCR assay which will be conducted according to procedures established in the test laboratory.

Throat swab 2 – Microbiome analysis

The second throat swab will be applied directly to an FTA card for DNA preservation, stored in a pouch with desiccant pack then frozen in a -80ºC freezer for future microbiome analysis.

Environmental samples

Mask portions and air sample cultures will be analysed for viable count or frozen at -80ºC freezer for future RTPCR.

11.3 Labelling of samples

Samples will be clearly identified with the study code, volunteer's unique anonymised identifier, sample ID and time point, and recorded in the study log. Samples will not be labelled with any personal identifiable information.

11.4 Microbiological sampling

Estimates of population density will be measured at each sampling point. Positive colonisation will be defined as the culture of at least one isolate from day 4 onwards after inoculation (Deasy, Guccione et al. 2015). We will record the duration of positive colonisation and the effectiveness of eradication therapy in clearing colonisation in 24 hours.

11.5 Immunological analyses

N. lactamica specific IgG will be measured using previously described methods. Cellular immunity will be assessed according to the procedures established in the test laboratories. Nasal fluid, collected by a nasosorption test, will be analysed for specific cytokine profiles. Nasal washes will be analysed for *N.lactamica* specific IgA and IgG. Saliva samples will be analysed for specific IgA. Other potentially relevant immunological assays may be performed on stored samples at the discretion of the investigator.

12. Assessment of safety

Safety of the volunteers will be assessed by analysing the frequency, incidence and nature of adverse events and serious adverse events arising during the study.

12.1 Definitions

Adverse Event (AE)

An AE is any untoward medical occurrence in a volunteer, including a dosing error, which may occur during or after administration of the inoculum and does not necessarily have a causal relationship with the intervention. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the study intervention, whether or not considered related to the study intervention.

Adverse Reaction (AR)

An AR is any untoward or unintended response to the inoculum. This means that a causal relationship between the inoculum and an AE is at least a reasonable possibility, i.e., the

relationship cannot be ruled out. All cases judged by either the reporting medical investigator or the sponsors as having a reasonable suspected causal relationship to the inoculum (i.e. possibly, probably or definitely related to the inoculum) will qualify as adverse reactions.

Unexpected Adverse Reaction (UAR)

An adverse reaction, the nature or severity of which is not consistent with the applicable information about the inoculum in the protocol, is considered as an unexpected adverse reaction.

Serious Adverse Event (SAE)

An SAE is an AE that results in any of the following outcomes, whether or not considered related to the study intervention.

- Death (i.e., results in death from any cause at any time)
- Life-threatening event (i.e., the volunteer was, in the view of the investigator, at immediate risk of death from the event that occurred). This does not include an AE that, if it occurred in a more serious form, might have caused death.
- Persistent or significant disability or incapacity (i.e. substantial disruption of one's ability to carry out normal life functions).
- Hospitalisation other than admission in the NIHR-WTCRF, regardless of length of stay, even if it is a precautionary measure for continued observation. Hospitalisation (including inpatient or outpatient hospitalisation for an elective procedure) for a pre-existing condition that has not worsened unexpectedly does not constitute a serious AE.
- An important medical event (that may not cause death, be life threatening, or require hospitalisation) that may, based upon appropriate medical judgment, jeopardise the volunteer and/or require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic reaction requiring intensive treatment in an emergency department or clinic, blood dyscrasias, or convulsions that do not result in inpatient hospitalisation.
- Congenital anomaly or birth defect.

Serious Adverse Reaction (SAR)

An adverse event (expected or unexpected) that is both serious and, in the opinion of the reporting investigator or sponsors, believed to be possibly, probably or definitely due to the inoculum or any other study treatments, based on the information provided in the protocol.

Suspected Unexpected Serious Adverse Reactions (SUSARs)

A SUSAR is a SAE that is unexpected and thought to be possibly, probably or definitely related to the inoculum.

12.2 Causality assessment

For each AE, an assessment of the relationship of the AE to the study intervention(s) will be undertaken. The relationship of the adverse event with the study procedures will be categorised as unrelated, unlikely to be related, possibly related, probably related or definitely related (Table 12.1). An intervention-related AE refers to an AE for which there is a possible, probable or definite relationship to the study intervention. The investigator will use clinical judgment to determine the relationship. Alternative causes of the AE, such as the natural history of pre-existing medical conditions, concomitant therapy, other risk factors and the temporal relationship of the event to the challenge will be considered and investigated.

0	**No Relationship**	No temporal relationship to the challenge ***and*** Alternate aetiology (clinical state, environmental or other interventions); ***and*** Does not follow known pattern of response to *N.lactamica*
1	**Unlikely**	Unlikely temporal relationship to the challenge ***and*** Alternate aetiology likely (clinical state, environmental or other interventions) ***and*** Does not follow known pattern of response to *N.lactamica*
2	**Possible**	Reasonable temporal relationship to the challenge; ***or*** Event not readily produced by clinical state, environmental or other interventions; ***or*** Follows expected pattern of response to *N.lactamica*
3	**Probable**	Reasonable temporal relationship to the challenge; ***and*** Event not readily produced by clinical state, environment, or other interventions ***or*** Follows expected pattern of response to *N.lactamica*
4	**Definite**	Reasonable temporal relationship to the challenge; ***and*** Event not readily produced by clinical state, environment, or other interventions; ***and*** Follows expected pattern of response to *N.lactamica*

Table 12.1: Guidelines for assessing the relationship of an AE to inoculation with *N. lactamica*

12.3 Reporting procedures for AEs

AEs will be recorded in the patient file and included in the annual safety report.

AEs that result in a volunteer's withdrawal from the study or that are present at the end of the study will be followed up (if volunteer consents to this) until a satisfactory resolution or stabilisation occurs, or until a non-study related causality is assigned.

12.3.1 Severity grading of clinical adverse events

The severity of clinical and laboratory adverse events will be assessed according to the scales in table 12.2.

GRADE 0	None
GRADE 1	Mild: Transient or mild discomfort (< 48 hours); no medical intervention/therapy required
GRADE 2	Moderate: Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required
GRADE 3	Severe: Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalisation possible

Table 12.2: Severity grading criterion for AEs.

12.3.2 Reporting procedures for serious AEs (SAEs)

In order to comply with current regulations on serious adverse event reporting to regulatory authorities, the event will be documented accurately and notification deadlines respected. SAEs will be reported to the Principal Investigator immediately when the study team is aware of their occurrence, as described in the relevant SOP. The external safety committee will be

notified of SAEs deemed possibly, probably or definitely related to study interventions; the sponsor will be notified immediately (within 24 hours) when the investigators are aware of their occurrence. SAEs will not normally be reported to the ethical committee(s) unless there is a clinically important increase in occurrence rate, an unexpected outcome, or a new event that is likely to affect safety of trial volunteers, at the discretion of the Chief Investigator. In addition to the expedited reporting above, the investigator shall include all SAEs in the annual Development Safety Update Report (DSUR) report.

12.3.3 Reporting procedures for SUSARs

The chief investigator will report all SUSARs to the ethical committee(s) within required timelines. The chief investigator will also inform all investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants. In addition, the chief investigator will report any SUSARs relating to licensed products used in the trial (ciprofloxacin) to the Medicine and Healthcare products Regulatory Agency (MHRA) using the electronic 'Yellow Card' System.

All SUSARs and deaths occurring during the study will be reported to the sponsor. For all deaths, any autopsy reports and relevant medical reports will be made available for reporting to the relevant authorities.

12.3.4 Procedures to be followed in the event of abnormal findings

Abnormal clinical findings from medical history, examination or blood tests, will be assessed as to their clinical significance. If a test is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains clinically significant, the volunteer will be informed and appropriate medical care arranged as appropriate with the permission of the volunteer. Decisions to exclude the volunteer from enrolling in the trial or to withdraw a volunteer from the trial will be at the discretion of the Investigator.

12.3.5 Foreseeable medical occurrences

The following medical occurrences are foreseeable:

- Local sensation effects in the nose following inoculation.
- Local bruises following venesection
- Adverse reactions to ciprofloxacin as detailed in the Summaries of product characteristics (SmPC)

12.3.6 Adverse events of special interest

Adverse events of special interest will be reported as SAEs. These are:

- Severe hypersensitivity reactions to the inoculum (e.g. anaphylaxis)
- Overdosing of the inoculum

12.4 Safety profile review

The safety profile will be assessed on an on-going basis by the investigators.

12.5 Study committees

12.5.1 External Safety Committee (ESC)

An external safety committee will be appointed prior to recruitment.

The role of the ESC is to provide overall supervision for the trial and provide advice through its independent Chair. The ultimate decision for the continuation of the trial lies with the Chief investigator following advice from the ESC.

The ESC will be responsible for reviewing and assessing this protocol prior to commencement of the trial, recruitment, interim monitoring of safety and effectiveness, trial conduct and external data. The ESC will first convene prior to trial initiation and will then define the frequency of subsequent meetings (at least annually).

All correspondence between investigator and ESC will be conveyed by the investigator to the trial Sponsor. The study protocol and implemented safety procedures will be discussed with the ESC before starting the study.

The chair of the ESC may be contacted for advice and independent review by the investigator or trial Sponsor in the following situations:

- Following any SAE deemed to be possibly, probably, or definitely related to a study intervention.
- Any other situation where the Investigator or trial sponsor feels independent advice or review is important.

12.5.2 Ethics Committee

The study will be reviewed by the HRA. A progress report will be submitted to the REC 12 months after the date on which the favourable opinion on which the form was given.

13. Analysis and Statistical considerations

13.1 Endpoints of the study

Primary endpoint

- Rise in serological specific antibody titre comparing day 0 versus day 14 and 28 days post eradication and comparing early (day 4) versus late (day 14) eradication

Secondary endpoints:

- Assessment of colonisation – culture of *N. lactamica* from throat swabs at day 4 for group 1 and days 4, 7 and 14 for group 2
- Assessment of eradication – culture of *N. lactamica* from throat swabs on the day of eradication, one day, 10 days and 28 days after eradication
- Rise in mucosal specific antibody titre comparing day of screening versus day 14 and 28 days post eradication and comparing early (day 4) versus late (day 14) eradication
- Change in nasal cytokine profile comparing day 0 versus day 4, 14 and 28 days post eradication and comparing early (day 4) versus late (day 14) eradication
- Difference in environmental shedding between short (group 1) and longer (group 2) periods of colonisation.

Safety endpoints:

- Occurrence of unsolicited adverse events within the study period
- Occurrence of serious adverse events within the study period

Safety analysis will be carried out for all volunteers that received the inoculum, regardless of whether or not they complete the study.

13.2 Sample size

The previous experimental *N. lactamica* challenge study showed a significant rise in serological antibody titre against *N. lactamica* over 2 weeks (Deasy, Guccione et al. 2015). This gave SDs on a log-10 scale of 0.11 for IgA saliva and 0.26 for serum total IgG. For this study, using the SD of 0.26 we will be able to confirm a 4 fold rise with 10 carriers of *N. lactamica* with 90% power using analysis of variance.

Allowing for a drop out rate of approximately 10%, we will therefore recruit volunteers until we have 11 individuals colonised for each group up to a maximum of 22 volunteers for each group. At a dose of 10^4 colony forming units (cfu), long lasting colonisation with *N. lactamica* is easily induced in 35-65% of participants. We showed that increasing the inoculum to 10^5 cfu increased the subsequent carriage of *N. lactamica* to 50% (Evans, Pratt 2011). Therefore approximately 44 individuals will be enrolled. Assuming a screening failure rate of 5%, we will need to screen approximately 46 potential participants to achieve colonisation in 11 of each group.

13.3 Statistical analysis

Statistical analysis will be performed by the study team and Dr Nick Andrews, Public Health England, Colindale. Differences between groups at each time point will be assessed using Fisher exact test and between time points within groups by McNemar test.

14. Study quality and Management procedures

14.1 Investigator procedures

Approved site-specific SOPs will be used at all clinical and laboratory sites.

14.2 Monitoring

Monitoring will be performed by the Sponsor according to International Conference on Harmonisation (ICH) Good Clinical Practice (GCP). Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements. The investigator sites will provide direct access to all trial related source data/documents and reports for the purpose of monitoring and auditing by the sponsor and inspection by local and regulatory authorities.

14.3 Study amendments

No amendments to this protocol will be made without consultation with, and agreement of, the Sponsor. Any amendments to the trial that appear necessary during the course of the trial must be discussed by the investigator and sponsor concurrently. If agreement is reached concerning the need for an amendment, it will be produced in writing by the chief investigator and will be made a formal part of the protocol following ethical and regulatory

approval (NRES-REC SOPs – Version 5.1 March 2012: http://www.hra.nhs.uk/wp-content/uploads/2013/08/NRES_SOPs_v5.1_2012.03.14.pdf).

An administrative change to the protocol is one that modifies administrative and logistical aspects of a protocol but does not affect the subjects' safety, the objectives of the trial and its progress. An administrative change does not require UK ethical committee or regulatory approval.

Any amendments to study documents will follow established HRA and REC requirements.

The investigator is responsible for ensuring that changes to an approved trial, during the period for which regulatory and ethical committee(s) approval has already been given, are not initiated without regulatory and ethical committee(s)' review and approval except to eliminate apparent immediate hazards to the subject.

14.4 Protocol deviation

Any deviations from the protocol will be documented in a protocol deviation form and filed in the site trial master file.

14.5 Quality Control, Quality Assurance and statuatory inspection

The UHS R&D department QA staff will provide Quality Assurance (QA) for the trial and perform internal audits to check that the trial is being conducted, data recorded, analysed and accurately reported according to the protocol, Sponsor's SOPs and in compliance with ICH GCP. The audits will also include laboratory activities according to an agreed audit schedule. The internal audits will supplement the sponsor's monitoring process and will review processes not covered by the sponsor's monitor.

A Quality control (QC) plan will be established at the start of the trial, as per local SOP.

The Sponsor, trial site and ethical committee may carry out audit to ensure compliance with the protocol, GCP and appropriate regulations. GCP inspections may also be undertaken by the regulatory authority to ensure compliance with protocol and national regulations. The sponsor will assist in any inspections.

14.6 Serious breaches

A serious breach is defined as "A breach of GCP or the trial protocol which is likely to affect to a significant degree – the safety or physical or mental integrity of the subjects of the trial; or the scientific value of the trial."

In the event that a serious breach is suspected the Sponsor will be informed as soon as possible and in turn will notify the REC and external safety committee within 7 days.

14.7 Study progress

The progress of the trial will be overseen by the Chief Investigator.

14.8 Study completion/termination

The trial will be considered complete upon the last volunteer/last visit at the site. The data will be sent to the sponsor in the timeframe specified in the Clinical Trial Agreement.

The study may be terminated early at the discretion of the Chief Investigator, Sponsor or External Safety Committee if there are safety concerns, concerns about compliance with GCP or other appropriate regulations, poor recruitment or new information becomes available which has an impact on the scientific validity or safety of the trial.

14.9 Exploitation and dissemination

The investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Findings will be published in peer reviewed journals as soon as possible, even where results prove negative. The authors will acknowledge that the study is funded by the NIHR Southampton Respiratory Biomedical Research Unit. The results of the study will be disseminated at relevant international scientific meetings. Volunteers will be sent a newsletter with a lay summary of the results of the study once the results are available. This will provide information regarding the study in general and not their individual results.

15. Ethics

15.1 Declaration of Helsinki

The Investigator will ensure that this study is conducted according to the principles of the current revision of the Declaration of Helsinki 2008.

15.2 ICH guidelines for good clinical practice

The Investigator will ensure that this study is conducted in full conformity to the ICH guidelines for GCP (CPMP/ICH/135/95) July 1996.

15.3 Informed consent

Written informed consent will be gained from all participants and their sleeping partners following the provision of detailed information about the aims of the study, the level of involvement required, and the risks involved. Participants will be provided with an information sheet prior to the start of the study either in print form or via email. They will be encouraged to use the contact details on this form to contact the research team to get further information if necessary. Prior to screening the participants' understanding of the study and risks involved will be explored and they will be asked to sign a consent form.

15.4 Informing participants' General Practitioners

A letter describing the study and the participant's involvement will be sent to their General Practitioner (GP) on the day of the screening visit. This will include contact details for the research team.

15.5 Research ethics committee

A copy of the protocol, proposed informed consent form, other written volunteer information and the proposed advertising material will be submitted to the REC and HRA for written approval, using the UK Integrated Research Application System. The investigator will submit and, where necessary, obtain approval from the REC for all subsequent amendments to the protocol and associated trial documents. A non-substantial amendment does not require UK ethical committee approval (NRES-REC SOPs – Version 5.1 March 2012: http://www.hra.nhs.uk/wp-content/uploads/2013/08/NRES_SOPs_v5.1_2012.03.14.pdf). The

investigator will notify deviations from the protocol or SAEs occurring at the site to the sponsor and will notify the REC of these if necessary in accordance with procedures.

15.6 Volunteer confidentiality

All data will be anonymised; volunteer data will be identified by a unique study number in CRF and database. Separate confidential files containing identifiable information will be stored in secured locations. Only the sponsor representative, investigators, the clinical monitor, the ethical committee(s) and the regulatory authorities will have access to the records.

16. Data handling and record keeping

16.1 Data handling

The chief investigator will be the data manager with responsibility for delegating the receiving, entering, cleaning, querying, analysing and storing of all data that accrues from the study in the site file held in the NIHR-WTCRF. The investigators will enter the data into the volunteers' CRFs, which will be in a paper format. This includes safety data, laboratory data (both clinical and immunological) and outcome data.

16.2 Record keeping

The investigators will maintain and retain appropriate medical and research records and essential documents for this trial in compliance with ICH E6 GCP and regulatory and institutional requirements for the protection of confidentiality of volunteers. The chief investigator, co-investigators and clinical research nurses will have access to records. The investigators will permit authorised representatives of the sponsor, regulatory agencies and the monitors to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress.

16.3 Source data and case report forms (CRFs)

All protocol-required information will be collected in CRFs designed by the investigator. All source documents, excluding hospital records, will be filed in the CRF. Source documents are original documents, data, and records from which the volunteer's CRF data are obtained. For this study these will include, but are not limited to; volunteer consent form, blood results, GP response letters, laboratory records and correspondence. In the majority of cases, CRF entries will be considered source data as the CRF is the site of the original recording (i.e. there is no other written or electronic record of data). In this study this will include, but is not limited to medical history, medication records, vital signs, physical examination records, urine assessments, blood results, adverse event data and details of study interventions. All source data and volunteer CRFs will be stored securely.

16.4 Data protection

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorised third party, without prior written approval of the sponsor.

17. Financing and insurance

17.1 Financing

The study will be funded by the NIHR Southampton Respiratory Biomedical Research Unit and will be supported by funding for Experimental Medicine by the National Institutes of Health Research through support from the Southampton NIHR Wellcome Trust CRF, the Wessex Comprehensive Research Network.

17.2 Insurance

The University of Southampton has a specialist insurance policy in place, which would operate in the event of any participant suffering harm as a result of their involvement in the research.

17.3 Compensation for time

Volunteers will be compensated for their time and for the inconvenience caused by procedures as below.

- Attending screening and follow-up sessions - £21 total (£15/visit plus additional £6 travel expenses)
- 1 x screening
- 1x challenge
- Up to 6 follow up visits

The maximum individual volunteers will be compensated is £168 and the minimum £15

If volunteers withdraw from the study prior to its completion they will be offered financial re-imbursement corresponding to the number of visits attended.

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