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Lactamica 7: Defining the immune response to nasopharyngeal colonisation by the commensal *Neisseria lactamica*

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Protocol Version 3.0 (04.10.2019)

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Version	Date	Authors	Modifications
1.0	29.03.2018	Adam Dale Anastasia Theodosiou	
1.1	06.04.2018	Adam Dale Anastasia Theodosiou	Changes to text where indicated to address peer review comments
1.2	26.04.2018	Adam Dale Anastasia Theodosiou	Changes to text where indicated by ERGO team.
1.31	09.07.2018	Adam Dale Anastasia Theodosiou	REC number added (title page & footer) Typo adjusted (pg. 13). $1x10^6$ cfu/ml changed to ~$2x10^8$ cfu/ml.
2.0	28.03.2019	Adam Dale	1. Screening Hb blood test changed from 5ml of blood to 3ml. 2. Replaced use of Freezerworks to custom database for tracking and tracing of biological samples throughout text. 3. Change in titles of study-specific SOPs mentioned. Note: no change to content of SOPs (i.e. administrative change only). 4. Change in recruitment affecting secondary outcome measure. No longer require recruitment of 10 *N. lactamica* and subsequently uncolonised individuals due to high colonisation rate (80%) demonstrated to date. 5. Added assessment for *N. lactamica* and *N. meningitidis* cross-reactive plasma and memory B-cells into secondary objectives

			and as a secondary outcome measure.
3.0	04.10.19	Adam Dale	1. Added Dr Fadil Bidmos and Professor Paul Langford as co-investigators (Imperial College London). 2. Outlined remit of Imperial College team. 3. Outlined strategy for transportation of volunteer PBMCs from the University of Southampton to Imperial College London.

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2.0 Introduction

2.1 *Neisseria meningitidis* in carriage and disease

Neisseria meningitidis (the meningococcus) is a commensal bacterial species of the human nasopharynx that has a rare propensity to cause invasive disease. Cross-sectional nasopharyngeal carriage surveys demonstrate that asymptomatic colonisation occurs in approximately 10% however carriage prevalence varies significantly with age, peaking at 20-30% in individuals in their late teenage years and early 20s (1). Despite this high carriage prevalence, the incidence of invasive meningococcal disease (IMD) in Europe is low at 0.68 cases per 100,000 per year (2). However, in the developing world, the incidence of IMD can be significantly higher. For example, in the African 'meningitis belt' epidemics of serogroup A disease occur where up to 1000 per 100,000 are affected (2).

The majority of IMD cases occur in infancy and early childhood (3 months - 2 years). A second smaller peak in IMD incidence is observed in individuals in their late teens and early 20s that coincides with the peak in meningococcal colonisation prevalence in this cohort. In Europe, the associated case fatality rate remains at 7.9% despite the provision of antibiotic treatment and supportive care facilities (3). Post-infective morbidity is significant in IMD survivors with over 50% suffering from severe physical sequelae (4).

2.2 Host immune interactions with the meningococcus

Asymptomatic colonisation with the meningococcus is an immunising event with complement-fixing serum bactericidal antibodies detectable following naturally-acquired colonisation within 2 weeks (5). In a landmark study published by Goldschneider *et al.* in 1969 it was established that a meningococcal strain-specific serum bactericidal activity (SBA) titre of <1:4 was associated with susceptibility to meningococcal disease in the setting of a serogroup C meningococcal outbreak amongst otherwise healthy military recruits. Importantly, in IMD cases, strain-specific SBA titres increased significantly on analysis of convalescent sera, suggesting that susceptibility to disease was associated with a baseline absence of SBA titre >1:4 as opposed to the inability to mount an appropriate SBA response (6).

Although a strain-specific SBA titre <1:4 is associated with susceptibility to IMD, this is not an absolute marker of disease susceptibility. This is highlighted in the study by Goldschneider *et al.* where, despite 35% of military recruits not having meningococcal SBA tires ≥4, the overall IMD attack rate in the outbreak setting was <1% (6). More recently, epidemiological and cross-sectional age-specific meningococcal SBA data demonstrate that the proportion of individuals with SBA titres ≥4 remains low (<10%) in children aged 2-15 where disease incidence is also low (7). These data support the possibility that factors other than SBA are likely important in protecting individuals from IMD. These factors could include the presence of additional protective immune mechanisms, protective microbial mechanisms, *e.g.* microbiome-related effects, or other host-specific biological effects (6).

Studies measuring cellular immune responses mounted following the acquisition of natural colonisation or infection with the meningococcus are limited compared to published studies relating to humoral responses. Pollard *et al.* demonstrated that proliferative responses of peripheral blood mononuclear cells (PBMCs) to meningococcal antigens, as measured by tritiated thymidine (^{3}H-TdR) incorporation in children 9.1 (median) weeks post-IMD, were significantly higher than responses from aged-matched controls (8). Cytokine profiles measured in the same subjects, as detected in meningococcal antigen-stimulated PBMC supernatants by enzyme-linked immunosorbant assay (ELISA), suggested that the T-helper phenotype was skewed towards T_H1 in the youngest children but T_H2 in older children. In a second study, Robinson *et al.* established that both Th1- and Th2-polarised CD4 T-cell responses were detectable in a longitudinal study where subjects naturally acquired meningococcal carriage over time (9).

2.3 Meningococcal vaccines: strengths, weaknesses and future directions

The work of Goldschneider *et al.* and others allowed for the assignment of an immune correlate of protection for strain-specific IMD (SBA titre ≥4 or a rise in titre by four-fold following vaccination) that provided an objective standard for *in vitro* studies of vaccine immunogenicity (6).

The first efficacious purified polysaccharide vaccines were developed in the 1960s at the Walter Reed Army Institute, USA, followed by bivalent and tetravalent (A&C and A, C, W-135 & Y, respectively) polysaccharide vaccines in the 1970s. Due to their polysaccharide composition, these vaccines depend on T-cell independent pathways to provide protection. They are poorly immunogenic in the population with the highest incidence of IMD, young children, and do not provide long-term protection (7).

The subsequent development of polysaccharide-conjugate vaccines, whereby meningococcal capsular polysaccharide is conjugated to a protein carrier, *e.g.* tetanus toxoid, proved hugely successful (10–12). Unlike their predecessors, meningococcal conjugate vaccines induce T-cell dependent immune responses that are primed for immunological memory and are highly immunogenic in infants (7). The nationwide introduction of the serogroup C conjugate meningococcal vaccine in the United Kingdom in 1999 resulted in a significant reduction in serogroup C IMD but also dramatically reduced serogroup C meningococcal carriage. The decrease in nasopharyngeal carriage following vaccination is the likely mechanism through which this vaccine induces herd immunity however the mechanism underlying this effect remains elusive (7,13–15). Understanding the immune mechanisms underlying this phenomenon has important implications for future vaccine development, specifically if future meningococcal vaccines are to be developed that prevent or eradicate the asymptomatic colonisation state.

The newly-developed polyvalent protein-based vaccine (Bexsero®) targeting serogroup B disease was introduced as a 2-dose regimen in infants in 2015. Pre-licensing immunogenicity studies demonstrated that good SBA titres were mounted against the majority of circulating UK serogroup B strains. In addition, early data following the roll out of Bexsero® was promising and demonstrated a 50% reduction in the incidence rate ratio in serogroup B meningococcal disease cases in the vaccine-eligible cohort when compared with the pre-vaccine period (16). However, there is no evidence that Bexsero® reduces serogroup B meningococcal carriage in vaccinated cohorts (17,18). If these observations are a true reflection of the population-wide situation, and serogroup B strains have similar transmission dynamics to those from serogroup C, we might predict that this vaccine may not be as effective as the conjugate serogroup C vaccine as the 'herd immunity' effect may be lacking.

To enable the development of new meningococcal vaccines or alternative strategies that protect against meningococcal carriage and generate herd immunity an improved understanding of the immune mechanisms conferring protection against meningococcal carriage is required. Understanding the immune mechanisms underlying carriage protection will enable the design of vaccines capable of inducing a response that protects against carriage.

2.4 Experimental human challenge with *Neisseria lactamica*

Neisseria lactamica is a commensal species of the human nasopharynx that preferentially colonises young children. Unlike the meningococcus, it is acapsulate and does not cause sporadic invasive disease in the context of the immunocompetent host. However, rare reports of *N. lactamica*-associated invasive disease are described in the setting of immunocompromised patient populations (19–25).

Data from numerous carriage studies demonstrate that an inverse relationship exists between nasopharyngeal colonisation with *N. lactamica* and *N. meningitidis* whereby, as colonisation with *N. lactamica* reduces with increasing age, meningococcal carriage increases (26,27). In addition, cross-sectional epidemiological data from the Faroe Isles has demonstrated that in the context of a serogroup B meningococcal outbreak across the islands, *N. lactamica* carriage prevalence was significantly higher on those islands where both meningococcal carriage prevalence and meningococcal disease incidence was lowest (28). Furthermore, in animal studies, a *N. lactamica* vaccine comprising whole killed bacterial cells or outer membrane proteins protected mice from fatal challenge in a bacteraemic meningococcal mouse model (29). Put together, these findings have led to speculation that *N. lactamica* colonisation may protect against *N. meningitidis* colonisation and the subsequent development of IMD.

The hypothesis that *N. lactamica* colonisation can protect against meningococcal colonisation was tested experimentally by Deasy *et al.* in a randomised, blinded, controlled human challenge study. The study demonstrated that in volunteers who were colonised with *N. lactamica* following intra-nasal challenge, meningococcal carriage was significantly inhibited. The observed effect was due to both displacement of existing meningococcal strains and inhibition of new meningococcal strain acquisition (30). Possible explanations for this effect are numerous and include: 1. inter-species bacterial killing; 2. competition for occupation of the nasopharyngeal niche/receptor binding and; 3. the possibility that cross-reactive immune responses primed or boosted by *N. lactamica* colonisation interact with *N. meningitidis* and eradicate colonisation or prevent new meningococcal acquisition events.

In 1978, Gold *et al.* demonstrated in a longitudinal study of healthy infants and children that only those who naturally acquired *N. lactamica* colonisation developed cross-reactive SBA to a meningococcal panel (26). However, this data must be interpreted with caution as participant numbers were small and bacteriological sampling time points infrequent which may bias the analysis. In a more recent controlled *N. lactamica* human challenge experiment conducted by Evans *et al.*, it was confirmed that colonisation with *N. lactamica*, like *N. meningitidis*, is an immunising event (31). In this study, experimentally-induced colonisation with *N. lactamica* resulted in a significant increase in *N. lactamica*-specific IgG (blood) and IgA (saliva) titres. Further analysis demonstrated significantly increased titres of cross-reacting opsonophagocytic antibody against a meningococcal panel in *N. lactamica*-colonised subjects but failed to demonstrate a significant rise in cross-reacting SBA. We conclude from this data that if cross-reactive immune responses are responsible for the observed phenomenon whereby *N. lactamica* colonisation prevents meningococcal colonisation, this is not due to production of cross-reactive SBA.

Understanding the mechanism(s) through which *N. lactamica* prevents meningococcal colonisation would allow for its potential exploitation to develop new strategies to protect against meningococcal disease. If a cross-reactive immune mechanism was to be elucidated this knowledge could be utilised to aid in the development of vaccines that induce immune responses that specifically affect colonisation. There is no evidence in the literature of direct competition *in vitro* between *N. lactamica* and *N. meningitidis* and mathematical models demonstrate that the protective effect of *N. lactamica* persists for 4 years, well beyond the period of natural colonisation by *N. lactamica* (32). Therefore, we hypothesise that an adaptive cross-reactive immune mechanism, independent of SBA, mediates *N. lactamica* protection against meningococcal disease.

Throughout the planned study we aim to use biological samples from volunteers enrolled onto a controlled *N. lactamica* human challenge study to assess the nature of the cellular immune responses induced upon colonisation with *N. lactamica* and to establish whether *N. lactamica*-specific responses cross-react with *N. meningitidis*.

2.5 Safety of *N. lactamica* in Human Challenge

N. lactamica is a normal coloniser of the nasopharynx in infants and young children and is not known to cause invasive disease in the immunocompetent host in the context of naturally-acquired

colonisation. We have demonstrated that experimentally-induced *N. lactamica* colonisation with strain Y92-1009 is safe having inoculated over 340 healthy adult volunteers with up to 10^5 colony forming units (cfu) to date in our portfolio of studies without any related adverse events (30,31).

2.6 General study overview

We plan to perform a controlled, volunteer blinded, *N. lactamica* human challenge study to enable *N. lactamica*-specific cellular immune responses to be outlined in detail. In addition, we aim to determine whether responses directed towards *N. lactamica* are cross-reactive with *N. meningitidis.*

Following enrolment onto the study, non-meningococcal carriers (as determined by microbiological culture of nasopharyngeal wash and retropharyngeal swab) will be challenged intra-nasally with either 10^5 cfu of *N. lactamica* wild-type strain Y92-1009 (30,31) suspended in sterile phosphate buffered saline (PBS), as used in previous studies, or PBS alone (control group). Following inoculation on Day 0, biological samples (nasal wash, nasal secretion, throat swabs and blood) will be taken from all volunteers on days +7 (+/-3), +14(+/-3) and +28(+/-5) post-challenge. On the day of inoculation (Day 0) the biological samples listed will be taken except for the nasal wash.

N. lactamica-specific B-cell and CD4+ memory T-cell responses will be measured in blood using a selection of *in vitro* assays and the results compared longitudinally in *N. lactamica* challenged and colonised *vs.* control challenged subjects. These experiments will establish the nature of T-cell and B-cell memory responses and plasma B-cell responses induced in response to *N. lactamica* colonisation and will determine if these responses are cross-reactive with *N. meningitidis*. Mucosal immune profiling and microbiome analyses will be performed on nasal secretion and nasal bacterial samples, respectively. Any remaining biological samples (following experiments performed to meet the current study objectives) will be transferred to our registered human tissue bank to enable future studies following additional ethics approval by the relevant bodies. These studies will depend on the result of the current study but may include specific studies of volunteer DNA, *e.g.* transcriptomic studies to understand the role of genes involved in the immune response. Additional institutional and ethical approvals will be sought for these studies if they become necessary.

3.0 Objectives:

3.1 Primary Objectives

1. To measure host T-cell and B-cell memory responses and plasma B-cell responses in blood to experimentally-induced nasopharyngeal colonisation with *N. lactamica*.

3.2 Secondary Objectives

1. Determine if *N. lactamica*-specific CD4+ T-cell memory responses and plasma and memory B-cell responses detected in blood cross-react with *N. meningitidis*.

2. To obtain nasal secretion samples and nasal bacterial samples from study volunteers at the prescribed time points to enable mucosal immune profiling and microbiome analyses to be performed.

4.0. Description and justification of the study design

4.1 Overview

We will perform a controlled, volunteer blinded, *N. lactamica* human challenge study that will enable *N. lactamica*-specific cellular immune responses in blood to be delineated. Nasal secretion and throat swabs will be taken to enable mucosal immune profiling and microbiome analyses to be performed. Subjects will be screened for nasopharyngeal *N. meningitidis* carriage -28 to -7 days (visit 1) and non-*Neisseria* spp. carriers will be challenged with either 10^5 cfu *N. lactamica* suspended in PBS or PBS only control (day 0, visit 2). Volunteers identified as carriers of *N. meningitidis* or *N. lactamica* at visit 1 will be excluded from the study and will not progress to experimental human challenge. Biological samples (nasal secretion, microbiome swab and blood) will be collected from volunteers who progress to the challenge (visit 2, Day 0) and on days +7 (+/-3), +14 (+/-3) and +28 (+/-5) post-challenge. Nasal wash sampling will not be taken at the challenge visit (Day 0) but will be taken on days +7(+/-3), +14 (+/-3) and +28 (+/-5) post-challenge. Any remaining biological samples (following experiments performed to meet the current study objectives) will be transferred to our registered human tissue bank to enable future studies following additional ethics approval by the relevant bodies.

4.2 Study volunteers

Healthy, non-smoking volunteers aged 18-45 years will be recruited (see section 15.2 for sample size details and power calculation). Volunteers who progress to experimental challenge will be randomised in a ratio of 2:1 to receive either 10^5 cfu *N. lactamica* intra-nasally or PBS control.

4.3 Blinding and randomisation

Volunteers will be blinded to the inoculum type received (*i.e. N. lactamica* or PBS control) throughout the study duration. Non-*Neisseria* spp. (*N. meningitidis* or *N. lactamica*) carriers will randomised in a ratio of 2:1 to receive either 10^5 cfu *N. lactamica* or PBS control using randomisation software, *e.g.* Sealed envelope™ available online.

4.4 Challenge procedure

Throughout the study our primary concern will be to ensure volunteer safety. As previously mentioned, *N. lactamica* is a commensal of the nasopharynx and is not pathogenic in the immunocompetent host. In previous *N. lactamica* human challenge experiments that utilised the same *N. lactamica* strain and inoculum dose we encountered no safety concerns. Taking this evidence together, we consider *N. lactamica* challenge to be safe.

4.5 Duration of volunteer participation

Volunteers will remain enrolled onto the study until they have completed their final visit, or up to 90 days from *N. lactamica* or PBS control inoculation, whichever is earlier.

4.6 Definition of the start and the end of the study

The start of the study is defined as the date upon which recruitment activity for the study begins. The end of the study is defined as 5 years after the date of the last visit of the last volunteer to allow for sample processing and data analysis.

4.7 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.

4.8 Involvement of the National Health Service (NHS) Research Ethics Committee (REC)

This study protocol has been submitted to the NHS REC for approval of this human challenge study. The study will only commence with approval from an NHS REC, and approval will be sought for any amendments prior to implementation.

5.0 *N. lactamica* Inoculum

5.1 Selection of the *N. lactamica* strain

Wild-type *Neisseria lactamica* (strain Y92-1009, sequence type 3493, clonal complex 613) will be used for this human challenge experiment. This strain is identical to that utilised in our previous challenge experiments (>350 volunteers to date) (30,31).

5.2 Production of the inoculum

Stocks of *N. lactamica* (strain Y92-1009, sequence type 3493, clonal complex 613) in Frantz medium containing 30% (v/v) glycerol have been previously prepared using the Good Manufacturing Practices pharmaceutical manufacturing facilities at Public Health England (Porton Down, United Kingdom). The stock utilised for this study is derived from the same batch as was utilised for our previous and currently ongoing human challenge experiments. Vials of ~$2x10^8$ cfu bacteria, suspended in Frantz medium containing 30% (v/v) glycerol, will be stored at -80°C in a dedicated human challenge freezer located within the NIHR-CRF laboratories. Access to this freezer will be controlled and limited only to trained study team members and a removal log maintained. Vials will be thawed and diluted in PBS to a final concentration of 10^5 cfu/ml within a dedicated decontaminated class 2 microbiological safety cabinet located within the NIHR-CRF laboratory following the study-specific standard operating procedure (SOP): Preparation and monitoring of *N. lactamica* inoculum for human challenge studies. This procedure will require 2 technicians to independently verify and record that the correct stock vial of *N. lactamica* has been selected and that it has been processed in line with the SOP to derive the final concentration of 10^5 cfu/ml *N. lactamica*. 1ml of this suspension will then be inoculated into the volunteer's nose, 0.5ml per nostril.

5.3 Quality assessment of the inoculum

Frozen stocks will be assessed for viability and contamination using standard microbiological procedures in line with SOP: Preparation and monitoring of *N. lactamica* inoculum for human challenge studies. Stocks will be checked monthly for changes in viable counts and the exact *N. lactamica* inoculum dose delivered to each volunteer documented and monitored in line with SOP: Preparation of

N. lactamica inoculum, SOP: Administration of *N. lactamica* inoculum, and SOP: Calculation of N. lactamica inoculum viable counts.

5.4 Transport of the inoculum

Previously transferred vials of the inoculum (*N. lactamica*, strain Y92-1009, sequence type 3493, clonal complex 613) are available for use in this study and already located at the University of Southampton. If further vials are required they will be transferred to the University of Southampton or University Hospital Southampton NIHR-CRF under temperature-controlled conditions. The aliquots will be placed into a container as secondary packaging. They will be placed inside leak and shock resistant transport boxes with secured lids. They will be sent by specialist courier from Public Health England and received by a member of the research team to ensure safe handling and storage.

5.5 Storage of the inoculum

The cell banks will be stored at -80^{o}C in a locked, dedicated, temperature-monitored freezer.

5.6 The optimal and safe dose of the inoculum

Our previous *N. lactamica* human challenge experiments demonstrated that approximately 50% of volunteers become colonised within 1-2 weeks following challenge with an inoculum of 10^5 cfu and that this inoculum dose is safe (30,31). This inoculum dose is optimal for the planned study and associated *ex-vivo* laboratory experiments as it will result in approximately equal numbers of *N. lactamica* colonised and uncolonised volunteers post-*N. lactamica* challenge. Please refer to sections 15.2 and 16.0 for details relating to volunteer recruitment and biological sampling targets derived from our sample size calculation.

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

After the inoculum is administered a sample of the residual inoculum will be diluted and cultured overnight. The dose will then be evaluated by viable count using the methodology outlined by Miles & Misra (33) and recorded.

6.0 Recruitment of study volunteers

6.1 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 a NHS REC 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 website).
- As a post on a Twitter, Facebook or Gumtree account owned and operated by our group.
- Video message posted on the 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 study at the NIHR-CRF, Southampton.
- On stalls or stands at exhibitions or fairs.
- Via presentations (*e.g.* presentations at lectures or invited seminars).
- Direct mail-out by the NIHR-CRF team: 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. 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-CRF 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.

For details relating to predicted volunteer recruitment numbers and rate of recruitment see section 15.2: Sample Size.

6.2 Volunteer information sheet

A volunteer information sheet will be sent electronically or given to the volunteer following pre-screening and at least 24 hours before the screening visit (visit 1). 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.

7.0 Screening (Visit 1, Days -28 to -7)

Individuals who have expressed an interest in taking part in the study who have previously been provided with an information sheet will be invited to attend a screening visit after a short telephone pre-screening. During the screening visit the study will be explained and if the volunteer has any questions they will be addressed. The screening visit will take place between days -28 to -7 prior to day 0 (day 0 defined as the day of intra-nasal challenge with *N. lactamica* or PBS). Volunteer eligibility to enrol will be ascertained by ensuring that they meet the inclusion criteria and do not meet any of the exclusion criteria.

7.1 Inclusion and exclusion criteria

7.1.1 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 study.
- For females only, willingness to practice continuous effective contraception (see below) during the study and negative pregnancy test at visit 1 (screening).

7.1.2 Exclusion criteria

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

- Active smokers.
- *N. meningitidis* or *N. lactamica* detected following culture of 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 studies/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 studies/trials investigating meningococcal vaccines or experimental challenge with *N. lactamica.*
- Use of oral or intravenous antibiotics within the period 30 days prior to the challenge.
- Any confirmed or suspected immunosuppressive or immunocompromised state, including HIV infection, asplenia, history of recurrent severe infections or use (more than 14 days) of immunosuppressant medication within the past 6 months (topical/inhaled steroids are allowed).
- Use of immunoglobulins or blood products within 3 months prior to enrolment.
- History of blood donation within the past 12 weeks for male volunteers, or 16 weeks for female volunteers.
- Allergy to yeast extract.
- 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.

7.1.3 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 volunteer
- True abstinence when this is in line with the preferred and usual lifestyle of the volunteer

7.2 Informed consent

Eligible 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 study, 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 without reason.
- 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). TOPS registration is mandatory for this study to prevent over-volunteering. Specific written consent for this will be sought from volunteers using the study consent form. The volunteer's National Insurance number +/- passport number will be required for this purpose (these data will be kept in the CRF and stored for 15 years in a secure location and then destroyed as outlined in section 19.1).
- 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 study, 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-CRF.

7.3 Medical history

Once fully informed written consent is obtained from the volunteer a brief clinical history will be taken. This will include details relating to:

- Demographics – age, DOB, gender.
- Past medical history – history of haematological disorders, previous meningitis, significant illness or immunocompromising conditions.
- Current medication history.
- Current illness.
- Allergies.
- Vaccination history.
- Recent antibiotic use.
- Sleeping partner.
- Household contacts.

A physical examination will only be performed where clinically necessary to determine participant suitability for enrolment.

7.4 Screening investigations

The following biological specimens will be taken from each volunteer by the study nurse(s) or doctor(s) at the screening visit:

1. For female participants, a negative pregnancy test will be required to proceed in the study.

2. A 3ml blood specimen will be taken to establish a baseline haemoglobin (Hb) concentration reading for each volunteer (required to ensure it is safe for enrolled volunteers to donate a total of 403ml of blood over the study period. This sample will be processed within the clinical haematology laboratories at UHS in line with UHS NHS FT procedures and policies). Please note that this is less than a single unit of blood (up to 470ml) donated by United Kingdom blood donors on a single day at NHS Blood and Transplant donation centres.

3. 1x posterior pharyngeal swab to determine Day 0 *Neisseria* spp. carriage status will be taken according to SOP: taking throat swab samples and SOP: processing of throat swab samples for Neisseria spp. culture.

4. 1x posterior pharyngeal swab to be utilised for microbiome analysis will be taken according to SOP: taking throat swab samples and SOP: Processing of throat swabs for microbiome analysis.

   A nasosorption test will be performed according to the SOP: Taking nasosorption samples to collect nasal fluid for assessment of mucosal immune responses.

5. A nasal wash will be taken (complementary to posterior pharyngeal swabbing) to determine Day 0 *Neisseria* spp. carriage status and for microbiome analysis according to SOP: Taking nasal wash samples.

8.0 *N. lactamica* or control (phosphate buffered saline) challenge (Visit 2, Day 0)

8.1 Study site

Intra-nasal challenge with 10^5 cfu *N. lactamica* suspended in PBS or PBS control will take place in the NIHR-CRF at the University Hospital Southampton NHS Foundation Trust (UHS NHS FT).

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 +/- Immediate Life Support (ILS) trained nurses will be present at inoculation and available within the NIHR-CRF for the post-inoculation period of observation. A research clinician will be contactable by telephone whenever volunteers are present in the unit for follow up visits. The NIHR-CRF is situated in the UHS NHS FT and a resuscitation team and intensive care facilities are available. Volunteers will have a 24 hours, 7 days per week contact number to contact research clinician in case of any adverse reactions during the study.

8.3 Infection control

The research team will adhere to the UHS 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 (CRF) and the label on the inoculum.

8.4.2 Review prior to the challenge

Prior to any procedure taking place, volunteers will be asked if they have any questions about the study and if they agree to continue with the study. Eligibility will be reconfirmed before the challenge is conducted. Prior to challenge volunteers will be asked three questions:

1. Are they currently well?
2. Have they been unwell since the last visit (details will be recorded)?
3. If they are currently taking, or have taken, any oral antibiotics since the last visit?

Baseline vital signs (pulse, blood pressure, temperature and respiratory rate) will be taken. A clinical examination will be performed by a member of the medical team if deemed necessary based on the review of the volunteer's medical history and vital signs.

8.4.3 Biological sampling for laboratory investigations

Prior to intra-nasal challenge on Day 0, the following biological specimens will be obtained from each volunteer by the study nurse(s) or doctor(s):

1. Nasosorption test will be performed according to the SOP: Taking nasosorption samples to collect nasal fluid for assessment of mucosal immune responses.

2. 1x posterior pharyngeal swab to determine Day 0 *Neisseria* spp. carriage status will be taken according to SOP: taking throat swab samples and SOP: processing of throat swab samples for Neisseria spp. culture..

3. 1x posterior pharyngeal swab to be utilised for microbiome analysis will be taken according to SOP: Taking throat swab samples.

4. 100ml of blood will be taken for use in *ex vivo* immunological assays.

Collection of all clinical samples will adhere to the UHS Standard Infection Control Precautions Policy (Version 5.0, 25/4/16). All biological sample processing will be performed on anonymised samples by

technical staff and the laboratory research team within the NIHR-CRF laboratory and the University of Southampton research laboratories.

8.4.4 Intra-nasal challenge

Preparing the inoculum

The inoculum will be prepared in the NIHR-CRF laboratory using a dedicated class 2 safety cabinet by trained laboratory 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 *N. lactamica* inoculum.

Time schedule of the challenge

The inoculation will take about 5 minutes, after which the volunteer will remain in the NIHR-CRF for further observation for a total of 30 minutes. Vital signs will be repeated following challenge.

Administering the inoculum

The challenge procedure will be carried out by one of the study doctors according to study specific SOP: Administration of *N. lactamica* inoculum.

9.0 Follow up visits (visits 3, 4 and 5 on days +7[+/-3], +14[+/-3] and +28[+/-5], respectively)

9.1 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 CRF and the label on the inoculum.

9.2 Review prior to biological sampling

Prior to any procedure taking place, volunteers will be asked if they have any questions about the study and if they agree to continue with the study. Prior to biological sampling volunteers will be asked three questions:

1. Are they currently well?
2. Have they been unwell since the last visit (details will be recorded)?
3. If they are currently taking, or have taken, any oral antibiotics since the last visit?

Vital signs will be taken and a clinical examination performed by a member of the medical team if they are deemed necessary based on the review of the volunteer's medical history. If there is any concern that a female volunteer may be pregnant a repeat point of care pregnancy test will be taken prior to continuation to biological sampling for the visit.

9.3 Biological sampling

Volunteers will undergo the following procedures (see table 12 for further details) at visits 3, 4 and 5 to obtain biological samples for planned laboratory experiments:

1. Nasosorption test will be performed according to the SOP: Taking nasosorption samples, to collect nasal fluid for assessment of mucosal immune responses.

2. 1x posterior pharyngeal swab to determine *Neisseria* spp. carriage status will be taken according to SOP: Taking throat swab samples.

3. 1x posterior pharyngeal swab to be utilised for microbiome analysis will be taken according to SOP: Taking throat swab samples.

   A nasal wash will be taken (complementary to posterior pharyngeal swabbing) to determine *Neisseria* spp. carriage status and for microbiome analysis according to SOP: Taking nasal wash samples, and SOP: Processing nasal wash for Neisseria spp. culture and microbiome analysis.
4.
5. 100ml of blood will be taken for use in *ex vivo* immunological assays.

10.0 Potential adverse events

10.1 Inoculation with *N. lactamica* or control (phosphate buffered saline)

The inoculation with 0.5ml of *N. lactamica* suspension or PBS control 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.

10.2 Phlebotomy

The maximum volume of blood drawn over the study period (405ml) 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.

10.3 Nasosorption, posterior pharyngeal swab 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.

11.0 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:

- Bacterial culture positive for *Neisseria* meningitidis or *Neisseria lactamica* following processing of posterior pharyngeal swab and nasal wash sample for microbiological culture following screening visit (visit 1).
- Blood Hb concentration below the normal laboratory reference range (on analysis of blood sample from screening – visit 1).
- 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 CRF. 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-study related causality has been assigned. Any volunteer who withdrew consent or is withdrawn from further study procedures may be replaced.
- Biological samples will be stored in a linked-anonymised format and given a unique identifier using a dedicated study database to be stored on password-protected University of Southampton/University Hospital Southampton computer networks. If a volunteer withdraws from the study, biological samples collected before their withdrawal from the study will be used/stored unless the volunteer specifically requests otherwise. The dedicated study database allows for tracking and tracing of study samples and will enable samples to be located and destroyed should the participant specifically request this. Anonymised data from volunteers withdrawn from the study will be included in the analysis of results relating to the study objectives.
- In all cases of volunteer withdrawal, excepting those of complete consent withdrawal, long-term safety data collection will continue as appropriate if subjects have received the inoculum.

12. Table 1 - Activities to be performed at each visit

	Screening (visit 1)	Inoculation (visit 2)	Follow up (visits 3, 4 & 5)		
Timeline (days)	**-28 to -7**	**0**	**+7 (+/-3)**	**+14 (+/- 3)**	**+28 (+/- 5)**
TOPS confirmation	+				
Volunteer Information Sheet	+				
Informed consent	+				
Medical history	+				
Vital signs		+			
Physical examination	(+)	(+)	(+)	(+)	(+)
Pregnancy test (females only)	+	(+)	(+)	(+)	(+)
Intra-nasal inoculation with *N. lactamica* or PBS control		+			
Nasal wash	+		+	+	+
Throat swab x2	+	+	+	+	+
Nasosorption test	+	+	+	+	+
Baseline Hb (3ml blood)	+				
Blood donation (100ml)		+	+	+	+

+ Performed in all volunteers
(+) Performed only if clinically indicated

13.0 Laboratory procedures

13.1 Laboratory work

SOPs will be utilised to process nasal wash samples, posterior pharyngeal swabs and nasosorption samples. Investigators will conform to well established laboratory safety standards. A SOP will be followed to process blood samples from volunteers to ensure the standardised extraction of PBMCs required for the planned experimental *ex vivo* assays. Volunteer PBMCs will be utilised to develop and optimise assays that will enable the quantification of cellular immune components (including *Neisseria* spp.-specific CD4+ memory T-cells and plasma and memory B-cell responses) prior to and following intra-nasal challenge and subsequent colonisation with *N. lactamica*. Literature published in the context of other human challenge models, *e.g. Streptococcus pneumoniae*, demonstrates that detectable responses in blood differ in their frequency and intensity over time (34,35). In light of these findings, and given this is the first time an attempt has been made to measure cellular immune responses to *Neisseria lactamica* in the context of a human challenge experiment, we plan to take large volumes of blood (100ml) at 4 strategically placed study time points (days 0, 7, 14 and 28) to maximise the chances of detecting a longitudinal change in *N. lactamica* specific B-cell or T-cell frequency in blood following challenge.

13.2 Processing and storage of samples

Biological samples taken from study volunteers throughout this study are required to address the primary and secondary objectives. These samples will be processed and utilised in planned experiments in an anonymised format (identified by unique study number only) within the NIHR-CRF laboratories and within the laboratories of Professor Robert Read (LC69 and LC71, C Floor, South Academic Block, University Hospital Southampton, University of Southampton) and Associate Professor Marta Polak (LE62, E Floor , South Academic Block, University Hospital Southampton, University of Southampton). Core research facilities available at the University of Southampton, *e.g.* the flow cytometry core (LF61, F Floor , South Academic Block, University Hospital Southampton, University of Southampton) and the tritiated thymidine facility (LF62, F Floor , South Academic Block, University Hospital Southampton, University of Southampton) will be used to gain experimental data.

Further research questions may become apparent throughout the course of our investigations that might only be addressed with biological samples obtained from human challenge study volunteers or indeed from specific volunteers enrolled onto this study. For these reasons, we intend to transfer any remaining biological samples from study volunteers (with specific volunteer consent) to a Human Tissue Authority (HTA) licensed tissue bank (the Southampton Research Biorepository, operating under the Faculty of Medicine's HTA licence) at the end of the study. This will enable future studies (including studies of volunteers DNA, *e.g.* specific studies to understand the role of genes involved in the immune response) to be performed (following additional scientific peer review and ethical approvals etc.) and will ensure the maximum amount of experimental information is obtained from these samples which, having been derived from a human challenge experiment, are unique.

Blood samples

PBMCs will be derived from whole blood within laboratories located within the NIHR-CRF laboratories or the University of Southampton and stored in liquid nitrogen at -196ºC within a locked and secure unit. PBMCs will be derived from whole blood using density gradient centrifugation in line with a dedicated SOP: Isolation of human PBMCs from whole blood . The investigatory team includes two expert immunologists (Dr Marta Polak, Associate Professor and Systems Immunology Group Principle Investigator, University of Southampton and; Dr Andrew Vaughan, Immunology Research Fellow, University of Southampton). These investigators will provide training where necessary and ensure that sample processing and analysis is only performed by staff who are trained in the laboratory procedures for this study and Good Clinical Practice. Any remaining PBMCs from study volunteers (with specific volunteer consent) will be transferred to a Human Tissue Authority (HTA) licensed tissue bank (the Southampton Research Biorepository, operating under the Faculty of Medicine's HTA licence) at the end of the study.

Nasal washes

Nasal wash fluid will be processed within laboratories located within the NIHR-CRF laboratories and the University of Southampton in accordance with SOP: Processing of nasal wash for Neisseria spp. culture and microbiome analysis. These specimens will be utilised to determine volunteer *Neisseria* spp. carriage status and for microbiome analysis. Any remaining nasal wash samples from study volunteers (with specific volunteer consent) will be transferred to a Human Tissue Authority (HTA) licensed tissue bank (the Southampton Research Biorepository, operating under the Faculty of Medicine's HTA licence) at the end of the study.

Nasosorption samples

Nasosorption samples will be processed within laboratories located within the NIHR-CRF laboratories and the University of Southampton in accordance with SOP: Processing of nasosorption samples. Any remaining nasosorption samples from study volunteers (with specific volunteer consent) will be transferred to a Human Tissue Authority (HTA) licensed tissue bank (the Southampton Research Biorepository, operating under the Faculty of Medicine's HTA licence) at the end of the study.

Throat swab 1 – bacterial culture

The first throat swab will be plated directly onto agar plates labelled with the volunteer number and visit number. The plates will be incubated and *Neisseria* spp. colonies identified using the SOP: Processing of throat swabs for Neisseria spp. culture. Positively identified *Neisseria* spp. will be sub-cultured onto fresh agar and individual colonies selected for indefinite storage.

Throat swab 2 – Microbiome analysis

The second throat swab will be processed in line with SOP: Processing of throat swabs for microbiome analysis. Any remaining throat swab samples from study volunteers (with specific volunteer consent) will be transferred to a Human Tissue Authority (HTA) licensed tissue bank (the Southampton Research Biorepository, operating under the Faculty of Medicine's HTA licence) at the end of the study.

13.3 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.

13.4 Microbiological sampling and identification of *Neisseria* spp.

Posterior pharyngeal swabs and nasal wash samples taken at the study time points will be cultured to assess for presence of *Neisseria* spp. in accordance with SOP: Processing of throat swabs for Neisseria spp. culture and SOP: Processing of nasal wash for Neisseria spp. culture and microbiome analysis. Positive colonisation will be defined as the culture of at least one *N. lactamica* colony from day +7 (visit 3) onwards after inoculation (30). In brief**,** swabs will be plated directly onto gonococcal selective (GC) agar and incubated at 37 ºC in 5% CO_2. Following incubation, colonies will be visually inspected within a class two microbiological safety cabinet and *Neisseria* spp. identified morphologically. Potential *Neisseria* spp. colonies will be screened for production of the beta-galactosidase enzyme (present in *N. lactamica* but not other *Neisseria* spp.) using X-gal (5-bromo-4chloro-3indolyl-beta-D-galactosidase, available from Sigma). Gram stain, the oxidase reaction and the API NH biochemical identification system (Biomerieux, France) will be utilised alongside other diagnostic microbiological testing methodologies (*e.g.* polymerase chain reaction assays) to provide a definite identification of *Neisseria* spp. If necessary, confirmation that recovered isolates are the inoculated strain can be determined on the basis of a unique RsrII restriction site which is absent from the pyruvate dehydrogenase E1 gene (pdhC) of *N. lactamica* Y92-1009 but present in the other 450 *N. lactamica* strains in the MLST database. *Neisseria* spp. isolates will be frozen in glycerol and stored at -80°C indefinitely to address current and future research hypotheses both at the University of Southampton and at collaborating institutions.

13.5 Immunological analyses

13.5.1 Investigation of the frequency and cross-reactivity of N. lactamica and N. meningitidis-specific CD4+ T-memory cells in blood

The frequency of *N. lactamica*- and *N. meningitidis*-specific CD4+ memory T-cells will be determined at day 0 and at post-challenge time points and results compared using statistical methods in challenge vs. control groups. A variety of immunological methodologies described in the literature, including the generation of CD4+ memory T-cell libraries, will be employed to achieve this goal (35–41).

The generation of CD4+ memory T-cell libraries from volunteers across study time points will be the primary method employed to determine the frequency of *N. lactamica* and *N. meningitidis*-specific T-cells. The associated methodologies are complex and are described in detail elsewhere (36,37). In brief, CD4+ memory T-cells will be purified using a magnetically-activated cell sorting CD4+ memory T-cell isolation kit (*e.g.* the kit available from Miltenyi Biotec). Purity of the enriched population will be confirmed by fluorophore-conjugated monoclonal antibody cell surface staining and flow cytometry prior to polyclonal expansion of CD4+ memory T-cells in 96-well format (*e.g.* by using CD3/CD28 activator beads [Dynabeads], available from ThermoFisher). Proliferative responses of individually-expanded CD4+ memory T-cell populations will be assessed by co-culturing expanded populations with autologous antigen-presenting cells (*e.g.* monocytes or lymphoblastoid B-cell lines) pulsed with outer-membrane vesicles (OMVs) of *N. lactamica* and *N. meningitidis* and control antigens (*e.g.* positive controls - staphylococcal enterotoxin B, phytohaemagglutinin and influenza haemaglutinin; negative controls - keyhole limpet haemocyanin and media unpulsed autologous monocytes alone) followed by assessment of T-cell proliferation using ^{3}H-TdR incorporation assay or Enzyme-Linked ImmunoSpot (ELISpot) assay (36,37).

Proliferating T-cell populations will be studied further to delineate their polarisation (*i.e.* Th1, Th2, Th17, Treg *etc.)* using, amongst other techniques, intra-cellular and surface-staining with fluorophore-conjugated antibody cocktails followed by flow cytometry (41). Assessment of *N. lactamica* specific CD4+ memory T-cell clone cross-reactivity with *N. meningitidis* (and vice versa, *i.e.* cross-reactivity of *N. meningitidis* CD4+ memory T-cell clones upon *in vitro* re-challenge with *N. lactamica*) will be performed using previously described methodologies (37,42).

Purified and enriched pulsed autologous monocytes will initially be utilised to activate expanded memory CD4+ memory T-cells. The longer term viability of these experiments will however be limited as only a finite number of monocytes will be available from the volunteer blood donation taken at each time point. In order to maximise the potential of these biological samples and perform experiments in the longer term it will be necessary to utilise an alternative autologous antigen presenting cell. To address this issue, we intend to develop Epstein-Barr Virus-immortalised lymphoblastoid cell lines from B-cells in donor blood for use in our current and future planned *in vitro* experiments. These cell lines are not deemed relevant material by the HTA and therefore will be held by our research team if necessary after the study is completed for further experiments.

13.5.2 Investigation of N. lactamica and N. meningitidis-specific B-cell responses in blood

The frequency of *N. lactamica*- and *N. meningitidis*-specific B-cells (plasma cells and memory B-cells) will be determined in blood in challenge *vs.* control volunteers at pre- and post-challenge time points using a variety of protocols outlined in the literature including the B-cell ELISpot assay (43). In brief, to detect antigen-specific B-cell responses using the ELISpot assay, PBMCs will be stimulated with a cocktail of polyclonal mitogens before measuring the number of antibody-secreting cells to *N. lactamica* or *N. meningitidis* OMVs and control antigens using an ELISpot plate reader (44,45).

B-cell data derived from the high-throughput B-cell assays performed at The University of Southampton will be utilised to select volunteer PBMC and plasma samples for further analysis by Dr Fadil Bidmos and Professor Paul Langford, members of the Molecular Infectious Diseases Group (MIDG), Imperial College London (St. Mary's campus), UK.

The experiments performed at Imperial College London will enable independent verification of the University of Southampton research teams' findings using techniques that enable the assessment of *N. lactamica*-specific B-cell cross-reactivity at the clonal level. If cross-reactivity is confirmed, additional experimental steps will be performed to identify the antigenic targets of the reactive B-cells. These techniques, collectively named 'reverse vaccinology 2.0' have recently been developed and validated for use by the Imperial College team (46, 47). Two study-specific SOPs will be utilised to ensure these experiments are carried out in a standardised manner: SOP: Isolation and single-cell sorting of *Neisseria*-specific plasmablasts from PBMCs and; SOP: Cloning of human monoclonal antibodies (hmAbs) from *Neisseria*-specific antibody producing cells. PBMCs will be transferred to Imperial College London as per the procedures set out in section 19.5 of the clinical study protocol.

The SOPs outlined above involve the expression of *Neisseria*-specific hmAbs in a Human Embryonic Kidney (HEK-293) cell line following the identification of *N. lactamica*-specific B-cells in volunteer PBMC samples. The resultant Mabs and host DNA from which they are transcribed are not deemed relevant material by the HTA and therefore will be held by the MIDG and the University of Southampton if necessary after the study is completed for further experiments.

14.0 Assessment of safety

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

14.1 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-CRF, 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.

14.2 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.

Table 2: Guidelines for assessing the relationship of an AE to inoculation with *N. lactamica*

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*

14.3 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.

14.3.1 Severity grading of clinical adverse events

The severity of clinical and laboratory adverse events will be assessed according to the scales in table 3.

Table 3: Severity grading criterion for AEs.

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

14.3.2 Reporting procedures for serious AEs (SAEs)

In order to comply with current regulations on SAE 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 study 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.

14.3.3 Reporting procedures for suspected unexpected severe adverse reactions (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 volunteers. In addition, the chief investigator will report any SUSARs relating to licensed products used in the study 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.

14.3.4 Procedures to be followed in the event of abnormal findings

Abnormal clinical findings from the 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 study or to withdraw a volunteer from the study will be at the discretion of the Investigator.

14.3.5 Foreseeable medical occurrences

The following medical occurrences are foreseeable:

- Local sensation effects in the nose following inoculation.

- Local bruises following venesection.

14.3.6 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.

14.4 Safety profile review

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

14.5 Study committees

14.5.1 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 study and provide advice through its independent Chair. The ultimate decision for the continuation of the study 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 study, recruitment, interim monitoring of safety and effectiveness, study conduct and external data. The ESC will first convene prior to study 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 study 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 study 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 study sponsor feels independent advice or review is important.

14.5.2 Ethics Committee

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

15.0 Analysis and statistical considerations

15.1 Endpoints of the study

Primary endpoint:

1. Change in frequency of *N. lactamica*-specific CD4+ memory T-cells, plasma B-cells and memory B-cells detectable in blood at day 0 vs. days +7, +14 and +28 in *N. lactamica* challenged and colonised vs. control-challenged volunteers.

Secondary endpoints:

1. Comparison of *N. lactamica*- vs. *N meningitidis*-specific CD4+ memory T-cell polarisation (*e.g.* Th1, Th2, Th17 *etc.*) in *N. lactamica*-challenged and colonised vs. control-challenged volunteers across study time points.

2. Comparison of the frequency of *N. lactamica*-specific CD4+ memory T-cells and plasma and memory B-cells that are cross-reactive with *N. meningitidis* in a sample of volunteers across study time points.

3. Comparison of the frequency of *N. meningitidis*-specific CD4+ memory T-cells and memory B-cells that are cross-reactive with *N. lactamica* in a sample of volunteers across study time points.

4. Comparison of nasal secretion cytokine profiles in *N. lactamica*-challenged and colonised vs. control-challenged volunteers across study time points.

5. Comparison of the nasopharyngeal microbiome in *N. lactamica*-challenged and colonised vs. control-challenged volunteers across study time points.

Safety endpoints:

1. Occurrence of unsolicited adverse events within the study period

2. 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.

15.2 Sample size

In the absence of data specifically assessing changes in the frequency of *N. lactamica*-specific CD4+ memory T-cells or memory B-cells following challenge, we have utilised serological data gathered from our previous challenge study as a surrogate to perform a power calculation. In our previous experimental *N. lactamica* challenge we demonstrated a significant rise in serological antibody titre against *N. lactamica* over 2 weeks (31). This gave SDs on a log-10 scale of 0.11 for IgA saliva and 0.26 for serum

total IgG. Using the SD of 0.26, we will be able to confirm a 4-fold rise in *N. lactamica*-specific IgG with 10 carriers of *N. lactamica* with 90% power using analysis of variance.

In order to avoid type 2 error and to have sufficient power to detect a difference in the frequency of *N. lactamica*-specific CD4+ memory T-cells and memory B-cells in blood following challenge we must therefore ensure full biological sample sets are obtained from:

1. At least 10 *N. lactamica* colonised volunteers following *N. lactamica* challenge.
2. At least 10 non-*Neisseria spp*. carrying (at any study time point) control-challenged volunteers.

We will continue to enrol participants to the study (using the 2:1 randomisation methodology outlined in section 4.2) until complete biological sample sets are obtained from participants as per groups 1 and 2 above. Based on our previous experience, we estimate that it will be necessary to enrol approximately 65 volunteers to reach the above targets (justification outlined in next paragraph). This number is difficult to predict accurately due to biological variation, particularly relating to *Neisseria* spp. carriage prevalence and volunteer drop-out rates. Hence, we may enrol fewer or more volunteers in total but, in any case, will cease enrolling volunteers once our targets are met.

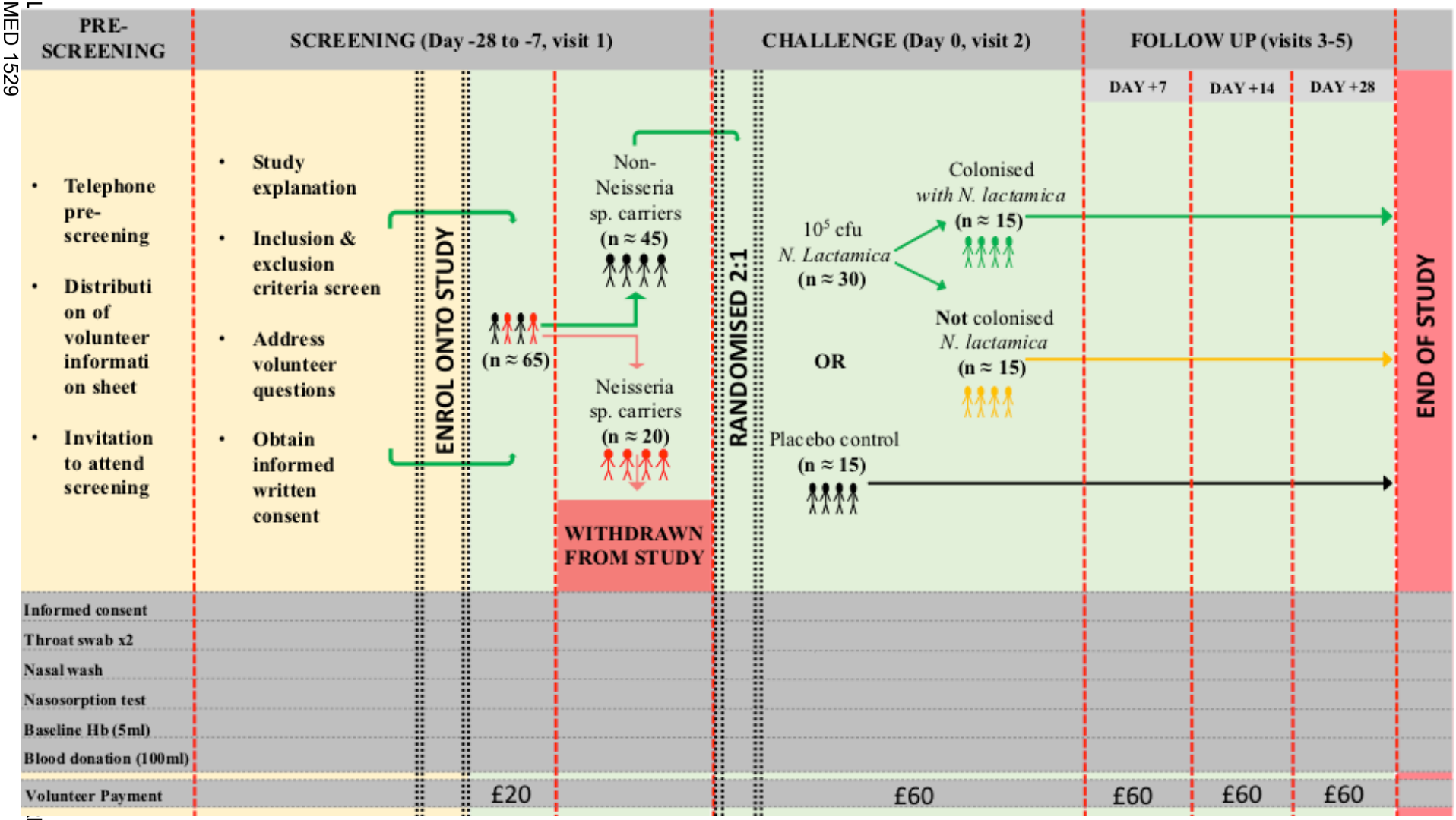
Working on the approximation of 65 volunteers enrolled, it is expected that up to 20 (30%) may carry *N. meningitidis* at baseline (screening visit 1, Days -28 to -7) (1). These volunteers will be withdrawn from the study prior to challenge (visit 2, day 0). The 45 remaining non-*Neisseria* spp. carriers will be challenged in a ratio of 2:1 with *N. lactamica* (n≈30) or PBS control (n≈15) on day 0. Based on our previous studies we predict that up to 50% of *N. lactamica*-challenged volunteers will become colonised (n≈15) (30,31). However, full biological sample sets are likely to be available from fewer than 15 *N. lactamica* colonised volunteers once volunteer drop-out (estimated at 20%) and biological variation are taken into account. In the PBS control-challenged group (n≈15) it is expected that there will be some natural acquisition of *N. meningitidis* (10-20%) between visit 1 (days -28 to -7) and visit 5 (day +28 +/- 5). In combination with volunteer drop out (estimated at 20%) it is therefore likely that fewer than 15 full biological sample sets will be available for analysis from the control-challenged group. Please refer to figure 1 for a diagrammatic representation of predicted volunteer sample size and pathway throughout the study period.

Based on the prediction of 65 enrolled volunteers required to meet our targets, we estimate that volunteer recruitment and associated biological sampling will take at least 9 months to complete.

15.3 Statistical analysis

Statistical analysis will be performed by the study team. The frequency of *N. lactamica*-specific CD4+ T-memory cells (within the memory CD4+ T-cell population) and the frequency of *N. lactamica*-specific plasma and memory B-cells will be determined across study time points in *N. lactamica*-challenged (and subsequently colonised) volunteers vs. PBS control-challenged volunteers. Differences in frequency between day 0 and days +7, +14 and +28 will be assessed for statistical significance using the paired t-test (normally distributed data) or Mann Whitney test (skewed distribution). For all analyses, a p value of <0.05 will be considered statistically significant.

16.0 Figure 1 – Volunteer recruitment, biological sampling and expenses payment diagram



17.0 Study quality and management procedures

17.1 Investigator procedures

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

17.2 Monitoring

The study Sponsor will oversee the monitoring that is conducted by the study team and the UHS Research and Development (R&D) department Quality Assurance (QA) team in line with NIHR-CRF protocols. The monitors will verify that the clinical study 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 study related source data/documents and reports for the purpose of monitoring and auditing by the sponsor and inspection by local and regulatory authorities.

17.3 Study amendments

No amendments to this protocol will be made without consultation with, and agreement of, the Sponsor. Any amendments to the study that appear necessary during the course of the study 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).

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 study and its progress. An administrative change does not require UK ethical committee or regulatory approval. Administrative changes will be notified to the Sponsor and any amendments to study documents will follow established HRA and REC requirements.

The investigator is responsible for ensuring that changes to an approved study, 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.

17.4 Protocol deviation

Any deviations from the protocol will be documented in a protocol deviation form and filed in the site trial master file. The Sponsor will be notified of any protocol deviations.

17.5 Quality control, quality assurance and statutory inspection

The UHS R&D department Quality Assurance (QA) staff will provide QA for the study and perform internal audits to check that the study 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 study, as per local SOP.

The Sponsor, study 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.

17.6 Serious breaches

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

In the event that a serious breach is suspected the Sponsor and external safety committee will be informed as soon as possible by the Chief Investigator. The sponsor will then inform the REC within 7 days.

17.7 Study progress

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

17.8 Study completion/termination

The clinical study will be considered complete upon the last volunteer/last visit at the site. The end of the study is defined as 5 years after the date of the last visit of the last volunteer to allow for sample processing and data analysis. 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 study.

17.9 Registration, exploitation and dissemination

The study will be registered on the ClinicalTrials.gov database. 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. Volunteers will be sent a copy of the paper once it is published and a lay summary will be made available to the study volunteers after manuscript publication. This will provide information regarding the study in general and not their individual results. The authors will acknowledge that the study is funded by the Wellcome Trust and supported by the NIHR-CRF. The results of the study will be disseminated at relevant international scientific meetings. This will provide information regarding the study in general and not their individual results.

18.0 Ethics

18.1 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.

18.2 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.

18.3 Informed consent

Written informed consent will be gained from all volunteers following the provision of detailed information about the aims of the study, the level of involvement required, and the risks involved. Volunteers 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 volunteers' understanding of the study and associated risks will be explored and they will be asked to sign a consent form.

18.4 Informing volunteers' General Practitioners

A letter describing the study and the volunteers' 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.

18.5 Volunteer confidentiality

Confidential files containing identifiable volunteer information will be stored in secured locations within the NIHR-CRF. Visit and laboratory source documents (pertaining to clinical activities and laboratory processing steps performed at each visit) will be labelled with unique study numbers. Members of the clinical study team (*i.e.* study doctors and nurses) will have access to confidential study files to enable linkage between study number and personal participant details and access to these files will be required during a study visits to confirm volunteer identity. A list of study participants and their unique identification numbers will only be available in the trial master file which will be accessible only by the clinical investigating team. Non-clinical researchers, *i.e.* individuals involved in laboratory processing steps and downstream experiments, will not be able to access personal volunteer data.

A database will be created into which visit and laboratory source document data will be entered. Data will be entered in a pseudonymised format identifiable only by unique study number. It will be compliant with General Data Protection Regulation (GDPR) and will only be accessible within the NIHR-CRF through secure, password-protected, NHS networks. Once the clinical study is complete and all source data is entered, this data will be interrogated to enable linked analysis with downstream experimental data (*e.g.* immunological assay data with clinical study data). Files obtained from the database for this purpose will be held in an anonymised manner on secure, password-protected University of Southampton networks accessible only by members of the investigative team.

Only the sponsor representative, investigators, the clinical monitor, the ethical committee(s) and the regulatory authorities will have access to the records.

19.0 Data handling and record keeping

19.1 Data handling and management

The chief investigator will be the data processor 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-CRF. The study will conform to University of Southampton Data Protection Policies and Guidelines.

The investigators will enter the pseudonymised data into the volunteers' CRFs, which will be in a paper format. This will includes safety data, volunteer microbiological laboratory data and outcome data. CRFs including personal volunteer data will be stored in secure locations within the NIHR-CRF. Selected non-personal data from volunteer CRFs will be entered into a database (as previously outlined in section 18.5) in a pseudonymised format and will only be accessible by the clinical research team within the NIHR-CRF through secure, password-protected, NHS networks.

Experimental laboratory data derived from pseudonymised volunteer biological samples performed at the University of Southampton will not be stored in CRFs but it will instead be stored in official laboratory workbooks or similar, or on password-protected networks at the University of Southampton, as is considered standard practice within academic scientific institutions. Official laboratory workbooks will be stored in locked offices and laboratories within the University of Southampton.

Experimental laboratory data derived from pseudonymised volunteer PBMC and plasma samples performed at the MIDG, Imperial College London, St. Mary's Campus, UK, will not be stored in CRFs. It will instead be stored in official laboratory workbooks or similar, or on password-protected networks at Imperial College London, as is considered standard practice within academic scientific institutions. Official laboratory workbooks will be stored in locked offices and laboratories within Imperial College London.

Pseudonymised clinical study data (following extraction from the database) with be analysed alongside experimental data using password-protected computers within the University of Southampton. Data submitted for final publication will be in the fully-anonymised format.

Data included within volunteers CRFs and the database will be stored for 15 years in line with UHS NHSFT archiving service following SOP: Standard Operating Procedure for the Archiving of Clinical Trial Data, SOP Number: R&D/Gen/Admin/011. Experimental data derived from downstream experiments on volunteer biological study samples will be stored within official laboratory workbooks in locked secure locations and on secure password-protected University of Southampton computer servers for 15 years. Additional backup copies of data will be stored on encrypted storage devices and kept within locked research offices at the University of Southampton.

19.2 Record keeping

The investigators will maintain and retain appropriate medical and research records and essential documents for this study 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.

19.3 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.

19.4 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.

20.0 Transportation of volunteer PBMC and plasma samples to the MIDG, Imperial College London, UK.

Experiments to be performed by the MIDG, Imperial College London, UK, will be focused on assessing for the presence of *N. lactamica*-specific B-cells and their cross-reactivity with *N. meningitidis* as outlined in section 13.5.2 of this protocol. This work will be performed using PBMCs and plasma collected from study volunteers using the previously-outlined study-specific SOPs.

PBMCs and plasma extracted from the blood of study volunteers will be stored in liquid nitrogen in cryovials having been labelled as per procedures outlined in section 13.3 of this protocol. These vials will be labelled with a unique volunteer number only and will not include any personally-identifiable information. Selected vials of PBMCs will be packaged by the University of Southampton research team and transported on temperatures of dry ice to the MIDG, Imperial College London, St Mary's Campus, UK, by recorded and tracked courier. Following receipt at Imperial College London, PBMCs will be kept in the secure, restricted-access MIDG research laboratories until they are utilised. All PBMC and plasma samples will be utilised before the NHS REC/HRA approvals expire.

21.0 Financing and insurance

21.1 Financing

The study will be funded primarily by the Wellcome Trust via. the Wellcome Trust Research Training Fellowship awarded to Dr Adam P. Dale and Professor Robert C. Read. In addition, central funding will be available from the Southampton NIHR-CRF to assist with housing overheads, administration and costs associated with research nurse assistance.

21.2 Insurance

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

21.3 Compensation for time

Enrolled volunteers will be compensated for their time and for the inconvenience caused by study procedures as below:

Visit 1 (screening) - £20
Visit 2 (inoculation) - £60
Visit 3 (follow-up) - £60
Visit 4 (follow-up) - £60
Visit 5 (follow-up) - £60

The maximum enrolled volunteers will be compensated is £260 and the minimum £20. If a volunteer is actively withdrawn from the study (*e.g.* due to carriage of *N. meningitidis* or low Hb at visit 1) or if they voluntarily withdraw from the study prior to its completion they will be offered financial re-imbursement corresponding to the number of visits attended. As volunteers will only be enrolled onto the study for a short period (approximately 4-6 weeks following intra-nasal *N. lactamica* challenge), a single payment will be made at the final volunteer visit or on a pro rata basis if study visits are missed for any reason.

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