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Serotype 3 Experimental Human Pneumococcal Challenge (EHPC); Dose ranging and reproducibility in a healthy volunteer population (Challenge 3)

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Recruitment status: currently recruiting and enrolling participants

ABSTRACT

Introduction

Since the introduction of pneumococcal conjugate vaccines (PCVs), pneumococcal disease rates have declined for many vaccine-type (VT) serotypes. However, serotype 3 (SPN3) continues to cause significant disease and is identified in colonisation epidemiological studies as one of the top circulating serotypes in adults in the UK. Consequently, new vaccines that provide greater protection against SPN3 colonisation/carriage are urgently needed. The Experimental Human Pneumococcal Challenge (EHPC) model is a unique method of determining pneumococcal colonisation rates, understanding acquired immunity, and testing vaccines in a cost-effective manner. To enhance the development of effective pneumococcal vaccines against SPN3, we aim to develop a new relevant and safe SPN3 EHPC model with high attack rates which could be used to test vaccines using small sample size.

Methods and Analysis

This is a human challenge study to establish a new SPN3 EHPC model, consisting of two parts. In the dose-ranging/safety study, cohorts of 10 healthy participants will be challenged with escalating doses of SPN3. If first challenge does not lead into colonisation, participants will receive a second challenge 2-weeks after. Experimental nasopharyngeal (NP) colonisation will be determined using nasal wash sampling. Using the dose that results in ≥50% of participants being colonised, with a high safety profile, we will complete the cohort with another 33 participants to check for reproducibility of the colonisation rate. The primary outcome of this study is to determine the optimal SPN3 dose and inoculation regime to establish the highest rates of NP colonisation in healthy adults. Secondary outcomes include determining density and duration of experimental SPN3 NP colonisation and characterising mucosal and systemic immune responses to SPN3 challenge.

Ethics and Dissemination

This study is approved by the NHS Research and Ethics Committee (Reference 22/NW/0051). Findings will be published in peer-reviewed journals and reports will be made available to participants.

ISRCTN registry number: 17879306. Registered 4th April 2022. Protocol version 3.0 (7th April 2022).

ARTICLE SUMMARY

Strengths and Limitations of the Study

- The use of a novel inoculation regime of a second (targeted booster) inoculation to participants not colonised after first challenge could increase colonisation rates, (unpublished data from ISRCTN12884329); higher colonisation rates will allow smaller sample sizes in future vaccine work, reducing study costs and time, resulting in improved, more globally accessible pneumococcal vaccines faster.

- Additionally, the use of clinical globally relevant strains from the UK and Malawi not used before in EHPC studies will allow the independent conduct of future pneumococcal vaccine studies in various settings and potentially a future global co-challenge collaborations network.
- The evaluation of bacterial shedding of SPN3 through hand swabs, cough plates and exhaled detection facemasks is novel and could provide greater understanding of how SPN3 is spread amongst individuals, eventually leading to improved infection and prevention control in hospitalised patients.
- Follow-up beyond 14 days will allow evaluation of longer-term local immune response to SPN3 and as well as providing longer term data on colonisation rate, density and duration.
- The main limitation of this study is the similarity to the EHPC Pneumo 1 study (1), which used different SPN3 isolates that are proprietary to a 3rd party to establish an SPN3 model.

INTRODUCTION

Streptococcus pneumoniae (SPN) is a major cause of morbidity and mortality from a lower respiratory tract infection globally(2). Available pneumococcal conjugated vaccines (PCVs) confer protection by reducing vaccine-type (VT) SPN colonisation density and are effective against invasive pneumococcal disease (IPD) (3). After the introduction of PCV-13 into national paediatric immunisation programs, overall combined-VT IPD amongst all ages significantly reduced (4). However, surveillance data showed limited declines in serotype 3 (SPN3) IPD in all ages, despite this serotype being included in PCV-13(5). This is thought to be due to specific characteristics of the SPN3 capsule(6), which could theoretically overwhelm the protective capacity of antibodies that are produced in response to the vaccine. There could be also other evasion mechanisms apart from antibody responses that could play a role in why SPN3 is different and continues to circulate amongst vaccinated communities.

An observational study of IPD rates in England 2014-2018 showed an increase in SPN3 IPD cases, with SPN3 contributing the most to total IPD deaths(7). Furthermore, a randomised controlled trial (RCT) did not show significant difference in SPN3 colonisation post PCV-13 in infants when compared to PCV-7 (which does not include SPN3) as a control (8). Taken altogether, current data suggests that even if PCV-13 protects against SPN3 directly to some degree, it does not sufficiently produce the sustained indirect protective effects seen against other VTs. Therefore, novel vaccines providing higher levels of protection against SPN3 colonisation (and therefore disease) are needed.

The Experimental Human Pneumococcal Challenge (EHPC) model allows vaccines to be tested for their effect on experimental SPN colonisation/carriage, in a more cost-effective manner than field studies, with fewer participants and shorter follow-up (9-11). Participants are intranasally inoculated with SPN, inducing a stable colonisation episode for about 1-3 weeks, at a density typical of natural colonisation. Host samples including nasal washes, nasal cells and blood are taken to assess colonisation as well as the immune responses. The model has provided key insights into human immune mechanisms that are associated with protection and susceptibility to colonisation acquisition (12-14). The model is well developed for serotypes SPN6B and SPN15B with over 2000 challenges in over 15 independent studies, showing the model is safe and has reproducible attack rates. In recent studies the model has been used to explore bacterial shedding and the transmission potential of SPN6B, demonstrating that hands can be a vehicle for transmission of SPN6B and lead to 18% colonisation when suspensions containing the bacteria are either sniffed from the hand or inserted into the nose via a finger (15). SPN transmission has been associated with living with a larger number of people, typically in prisons or nursing homes(16), therefore evaluating methods of shedding provides invaluable insight into how this transmission occurs and can be prevented.

More recently, 96 healthy participants were challenged with three different proprietary strains of SPN3 at various doses. Colonisation rates varied from 30-70% and the model was shown to be safe and feasible (1). Interestingly, there was no increase in levels of nasal SPN3 anti-capsular antibodies in colonised participants at day-14 post-inoculation, suggesting that there could be a lack of immunogenicity with SPN3, unlike has been demonstrated in previous studies(8). Additionally, 30.2% of participants reported symptoms when questioned at routine clinic visits, of whom the majority

described a sore throat. SPN has not previously been commonly associated with pharyngitis(17) and further investigation into this is required.

To ensure the EHPC model remains at the cutting edge of pneumococcal (current and future) vaccine assessment, we are proposing here to set up an EHPC model with carefully selected non-proprietary SPN3 strains and a second (targeted booster) inoculation to achieve maximum attack rates. In this study, in addition to determining the optimal dose and isolate of SPN3 to establish highest rates of colonisation in the human nasopharynx, we intend to improve the knowledge of both mucosal and serological immune responses to SPN3 colonisation. We will investigate longer-term immune response to SPN3 colonisation (beyond 14 days) and for the first time we will use a targeted booster SPN3 inoculation to improve colonisation rates and evaluate the impact that this has on immunogenicity. We hypothesise that this could better simulate natural SPN colonisation in high transmission settings, whereby individuals are likely to be repeatedly exposed.

Additional exploratory outcomes will be assessed, including evaluating SPN3 shedding and further investigating symptoms experienced post-inoculation. The results from this study will be used to inform development of improved SPN3 vaccines and to inform design of future pneumococcal vaccine RCTs. We plan to transfer this SPN3 model to Malawi, where SPN3 is a dominant disease-causing and antimicrobial resistance-transmitting serotype. Our previous transfer of the SPN6B model has been safe and successful, revealing important differences in vaccine response in highly endemic low resource settings (18).

The primary aim of this study is to determine the optimal SPN3 dose and isolate to establish experimental nasopharyngeal colonisation in healthy adults. Success in this project will result in:

1. Expansion of the EHPC model to demonstrate safe SPN3 colonisation with new non-proprietary isolates. This will allow better understanding of SPN3 colonisation dynamics and identification of correlates of protection.
2. Determination of the optimum isolate/dose and safety of SPN3 booster inoculation, to allow future testing of vaccines in double blind randomised controlled trials with even smaller numbers of participants than usual EHPC studies (and significantly less than are required for field studies).

METHODS AND ANALYSIS

Study Overview

This protocol has been designed using the SPIRIT reporting guidelines (19).

This is a human challenge study of healthy adult participants who will be nasally inoculated with well-characterised, fully sequenced-penicillin sensitive SPN3, for the assessment of acquisition of nasal pneumococcal colonisation and immune responses. We will conduct a dose-ranging study to determine the optimum SPN3 isolate and dose for safe colonisation acquisition and confirm the dose and safety in a subsequent larger cohort in a reproducibility study. This study will run from July 2022 to October

2023 at the Accelerator Research Building, Liverpool School of Tropical Medicine (LSTM), Liverpool, UK.

Figure 1 displays the study process. In the dose ranging study, sequential cohorts of 10 healthy participants will be challenged with escalating doses of SPN3. We will start at 10,000 colony forming unit (CFU)/naris and after n=10, escalate to 20,000 CFU/naris (n=10) and then 80,000 CFU/naris (n=10) if safe to do so. The first challenge cohort at 10,000 CFU/naris will start slowly with a smaller group (n=1-6) inoculated per week, for safety, before completing the group (n=10). We may increase the dose further depending on colonisation rates after consultation with the Trial Monitoring Group (TMG). If optimum attack/ colonisation acquisition rates are achieved (≥50%) in the lower doses, the higher dose escalation groups may not be completed - this will be discussed with the trial steering committee (TSC) before a decision to omit the higher doses is made. We may escalate the dose before a cohort of n=10 is inoculated, and subsequent escalated doses may be omitted if the colonisation rates are low. This is in the interest of not inoculating participants at a dose that could be futile. This will be at the discretion of the CI.

Up to 2 isolates will be tested in this manner. In case of not achieving the desired attack rate with any of the isolates, the data obtained will be reviewed to either test an additional isolate or complete the cohort at a lower attack rate.

Using the dose, isolate and inoculation regime that results in ≥50% colonisation acquisition/attack rate, with a high safety profile, we will complete the cohort with another 33 participants to check for reproducibility of attack rate. In previous research conducted with SPN6B, colonisation rates of 58.5% were seen with single inoculation and of those who were negative after the first inoculation, a further 41.7% then became positive after a second inoculation, resulting in a combined colonisation rate following two inoculations of 70.7%.

Study Objectives and Outcomes

Study objectives and outcomes measures are demonstrated in Table 1.

Table 1. Objectives and Outcome Measures

	Objectives	Outcome Measures
Primary	To determine the optimal SPN3 dose and isolate to establish colonisation of the nasopharynx in healthy adults using the EHPC model.	The proportion of participants with experimental SPN3 colonisation of the nasopharynx, determined by SPN3 presence in classical microbiological culture in at least one nasal wash (NW) sample, at any time point following one or two inoculations (combined and individually). This will be assessed for each isolate and dose separately.

Secondary	To ascertain the rate of experimental colonisation acquisition of SPN3 in healthy adults post challenge, by classical and molecular methods.	The rate of occurrence of SPN3 experimental colonisation of the nasopharynx, determined by SPN3 presence in classical microbiological culture and qPCR (combined and individually) from at least one NW sample at any time point following one or two inoculations (combined and individually).
	To determine the density of experimental SPN3 colonisation of the nasopharynx.	The bacterial density of experimental SPN3 colonisation of the nasopharynx in NW, at each and any time point following one or two inoculations (combined and individually), determined by classical microbiological culture and molecular methods.
	To determine the duration of experimental SPN3 colonisation of the nasopharynx.	The duration of experimental SPN3 colonisation of nasopharynx determined by the last NW sample following one or two inoculations (combined and individually) in which SPN3 is detected by classical microbiological culture or molecular methods.
Exploratory	To determine grading score of symptoms during experimental colonisation (e.g., sore throat, rhinitis, nasal congestion).	The presence of mild or moderate symptoms as recorded on a Likert scale in participants with SPN3 within the first 7 days after inoculations. Sore throat grading score will also be used if applicable.
	To characterise mucosal immune cell populations and dynamics in response to SPN3 experimental inoculation in nasal cell samples.	Cell immunophenotyping using flow cytometry methods to identify and characterise cell populations such as neutrophils, monocytes, T cells and B cells in nasal cells samples at screening and 2, 7, 13, 16, 21 and 28 days after first inoculation.
	To determine the level of mucosal and systemic SPN3 polysaccharide-specific antibodies at baseline and after SPN3 experimental inoculation.	Measurement of anti-SPN3 polysaccharide specific immunoglobulin G (IgG) levels in serum and nasal wash samples using ELISAs.
	To determine the levels of polysaccharide specific SPN3 memory B cells at baseline and after SPN3 experimental inoculation.	Quantification and characterisation of the number of SPN3- polysaccharide specific memory B cell populations in PBMC samples using flow cytometry methods at screening and 13 and 28 days after first inoculation.
	To describe nasal inflammatory kinetics induced by SPN3 experimental inoculation.	Measurement of 30 cytokines and chemokines using multiplex Luminex in nasosorption samples at screening and 2, 7, 13, 16, 21 and 28 days after first inoculation.

	To assess bacterial shedding after pneumococcal colonisation.	The rate of pneumococcal bacterial shedding as defined by swabs of hand and cough-plate based assessment post inoculation (presence and density CFU/ml) at 2, 7, 13, 16, 21 and 28 days after first inoculation. (During COVID-19 pandemic cough sampling may not be performed).
	To compare 'exhaled detection facemask' (EDF) with cough plate-based methods of assessing SPN3 bacterial shedding post-pneumococcal inoculation.	The rate of pneumococcal bacterial shedding as defined by exhaled detection facemask and cough-plate based assessment post- inoculation at 2- and 7-days post-inoculation (presence and density [CFU/ml])
	To determine the effect of natural SPN (non-SPN3) colonisation on SPN3 colonisation.	The rate of occurrence and density of SPN3 colonisation of the nasopharynx post-inoculation in natural SPN carriers, determined by SPN3 presence and density in classical microbiology culture or qPCR from at least one NW sample at any timepoint post-inoculation.
	To compare rates, density and duration of SPN3 colonisation in saliva vs nasopharyngeal samples.	The presence, density and duration of SPN3 colonisation in NW and saliva samples at any timepoint post-inoculation, identified using classical microbiology culture or qPCR

Study Participants

Inclusion/Exclusion Criteria

Healthy participants aged 18-50 (inclusive) who are fluent in English, have access to a mobile telephone and have capacity to give informed consent will be allowed to participate. This age range minimises the risk of IPD and allows comparison with our previously published EHPC work.

Tables 2 outlines the exclusion criteria for this study.

Table 2. Exclusion Criteria

Research participant	Currently involved in another study unless observational or non-interventional, excluding the EHPC bronchoscopy study and COVID-19 observation and interventional trials
	Participant in any previous EHPC trial in past 1 year
	Previous EHPC trial inoculated with SPN3 in last 3 years
Vaccination	Previous pneumococcal vaccination PPV23 or PCV13 or PCV10.
Allergy	Allergy to penicillin

Comorbidities	Chronic respiratory, cardiac, kidney, liver or neurological disease
	Connective tissue disease
	Diabetes
	Immunosuppressive disease
	Recurrent otitis media
	Asplenia or spleen dysfunction
	Cochlear Implants
	Major cerebrospinal fluid leak
	Uncontrolled medical/surgical conditions (at discretion of study doctor)
	Major pneumococcal illness requiring hospitalisation within the last 10 years
	Other conditions considered by clinical team as a concern for safety/integrity of the study
	Significant mental health problems (uncontrolled or previous admission to psychiatric unit)
Medications	Immunosuppressive medication
	Long-term antibiotic use or use of antibiotics in 28 days prior to inoculation
Direct caring role or close contact with individuals at higher risk of infection (during the EHPC period) unless wearing personal protective equipment	Children aged under 5
	Chronic ill health or immunosuppressed adults
	People that are part of extremely vulnerable group as defined by Public Health England
Smoking/drug/alcohol use	Current or ex-smoker (daily cigarettes/e-cigarettes/smoking of recreational drugs) in the last 6 months. Participants who smoke <5 cigarettes per week may be included
	Previous significant smoking history (>20 pack years)
	History or current drug or alcohol abuse (frequently drinking alcohol): men and women should not regularly drink >3 units/day and >2 units/day respectively) at discretion of the clinician
Biologically female participants of child-bearing potential who are currently pregnant/lactating, intending on becoming pregnant or not on effective birth control	
Overseas travel (involving air travel) planned in follow up period of study visits	
Participants who meet following criteria at time of screening: • Unexplained/concerning findings on history/examination • Haemoglobin <90g/dL • White cell count (WCC) <1.5 x10^9/l or >12 x10^9/l	

- Platelets <75 x10^9/l
- Oxygen saturations <94%

Additionally, we will employ temporary exclusion criteria, including:

- COVID-19 symptoms or confirmed current COVID-19 infection.
- Current/acute illness within 14 days prior to inoculation if COVID-19 negative.
- Positive COVID-19 swab within 10 days of inoculation. Participants will require negative lateral flow test prior to inoculation.
- COVID-19 vaccination 21 days prior to inoculation.
- Natural SPN3 colonisation identified in baseline nasal wash.

Participants who have been temporarily excluded at screening may be re-screened at a later date to assess their eligibility at this time for inclusion into the study.

Participant recruitment

Participants will be recruited from the general public, including through public engagement events, social media, generic research communication mailing lists, large local employers and local universities.

Participant Timeline

For both parts of the study, participants will attend an identical visit schedule, however the samples taken at these visits will differ (Figure 2). Participants will attend a screening visit, inoculation visit (day 0) and then follow-up visits at days 2, 7, 13 and 28. Figure 2 outlines the samples that are required at each visit.

All will first attend a screening visit to confirm eligibility through medical history, clinical examination and acquisition of samples including a full blood count and nasal wash sampling. Baseline nasal washes will be evaluated for natural colonisation with SPN (and serotype if present) through classical microbiological culture and molecular methods.

All participants will then attend an inoculation visit (day 0), where fluid containing SPN3 will be instilled into their nose. At this visit, they will be given a safety pack containing a thermometer, safety information leaflet and a 5-day course of amoxicillin. They will be instructed to contact the research team daily for 3-5 days with their temperature recording and any symptoms. Participants who report symptoms consistent with pneumococcal disease will be reviewed in person by a clinician and may be instructed to take their antibiotic course. Participants have 24-hour access to research clinicians as well as access to hospital facilities and prompt treatment if required.

In the dose-ranging study, a second targeted booster inoculation will be given to participants on day 14 if they have tested negative for SPN3 on day 2 and 7 samples. In the reproducibility study, it will be at the discretion of the chief investigator (CI) to decide if targeted booster inoculation should be applied, based on results of the dose-ranging study. If a second inoculation is included in the reproducibility study, it will be only given at day 14 to those participants who are negative for SPN3 at days 2 and 7

(these participants will be asked to attend additional follow-up visits at days 16 and 21 for further sampling).

During the dose-ranging phase, nasal wash samples pre- and post-inoculation will be collected to assess colonisation acquisition and density, by classical microbiological culture and molecular methods. Additionally, serum samples will be taken at baseline, 13 and 28-days after inoculation, to measure levels of anti-capsule polysaccharide immunoglobulin G (IgG). Nasal cell samples will be taken to characterise cellular populations and dynamics by flow cytometry.

During reproducibility phase, nasal wash and nasal cell samples will be collected as in the dose-ranging study. A viral swab will be collected pre-inoculation to test for viral co-infection. Nasal filters (nasosorption) will be collected pre- and post-inoculation to assess mucosal inflammation using 30-plex Luminex. Saliva samples will be taken pre- and post-inoculation to compare rates, density and duration of SPN3 colonisation in saliva and nasopharyngeal samples**.** Peripheral blood mononuclear cells (PBMCs) will be obtained at baseline, at 13-days and at 28-days post-inoculation. PBMCs will be used to characterise memory B cell and other immune cell populations using flow-cytometry based methods. Serum samples will be taken at baseline, 13 and 28 days after inoculation to measure levels of anti-capsule polysaccharide IgG. Exhaled detection facemasks (EDF) will be collected at day 2, to allow comparison against other shedding samples for detection of SPN3. A subgroup of participants who have demonstrated colonisation with SPN3 at day 2 will undergo EDF sampling at day 7.

At the end of the study, study participants who have been positive for SPN3 colonisation at any time point, and who have not subsequently had two consecutive negative nasal wash samples, will be asked to take oral amoxicillin 500mg three times daily for 5 days with the aim to clear / assist with clearing of colonisation.

Participant Retention

We will use an online booking system for appointments, to ensure that reminders are sent to participants for each appointment. There are windows of 2 days around all appointments to allow participants to move their appointments within window, if needed. Participants are remunerated on study completion.

Data Collection Methods

Samples will only be collected by staff members who are trained and delegated to do so. Table 3 describes the data collection methods.

Table 3. Data Collection Methods

Determination of colonisation	Colonisation will be defined as result of nasal washes taken at 2, 7, 13, 16, 21 and 28-days post-inoculation. Nasal washes will be performed using the Naclerio method (14), which is a validated technique to collect nasal bacterial specimens. Nasal washes will be plated onto culture media. Colonies will be confirmed as SPN3 using classical microbiological techniques. Results from the cultured nasal wash will also be confirmed using Polymerase Chain Reaction (PCR) based methods.

Molecular methods of determination of colonisation	DNA will be extracted from bacterial pellet post nasal wash sample centrifugation. SPN3 detection will be done by multiplex qPCR. This technique will enable us to detect individuals who are potential carriers with very low bacterial density. This multiplex qPCR is well validated in our laboratory (1).
Viral detection and quantification	Viral multiplex qPCR for detection and quantification will be performed on DNA and RNA of stored throat swab and/or nasal wash to detect all common respiratory viruses.
Mucosal and systemic immune responses	Serotype-specific responses and their association with both acquisition and clearance of colonisation (density and duration) will be measured. We will compare antibody levels and function between those colonised and those protected against colonisation. Levels of immunoglobulin to the capsular polysaccharides SPN3 in serum and nasal washes before and after inoculation will be determined. Levels of SPN3-specific memory B cells will be assessed using PBMCs collected pre and post inoculation. Flow cytometry will be used to examine the induction of antigen-specific cellular responses in blood including B cell and T cells. Mucosal cellular responses will also be measured by flow cytometry on nasal cell samples. Additionally, the mucosal inflammatory response associated with inoculation will be evaluated using 30-plex Luminex method to detect cytokines and chemokines in nasal filters

Sample Size Calculation

We have adopted a step-wise approach to escalating the inoculation dose. The protocol is designed to: a) minimise the possibility that we try repeatedly to attain colonisation at a dose in which it is unlikely to happen; b) maximise safety by inoculating small groups before continuing onto larger groups (in which we will have the statistical power to give reasonable precision of our estimate of colonisation rates).

Based on previous experience in studies of SPN6B and SPN3, we expect that with a colonisation rate of 45%, with 95% confidence level, and a margin of error of 15%, our study will be complete 43 participants with a single inoculation dose. Depending on colonisation rates at different inoculum doses of the SPN3 isolate, we need a minimum of 43 and a maximum of 93 participants to complete the study. This allows for up to two isolates to be taken through to the highest dose of 80,000 CFU/100µL. For example, if two isolates were tested at each dose in 10 participants in the dose ranging study, this would equate to 60 participants. A further 33 participants would then take part in the reproducibility study, equating to a total of 93 participants. To ensure that we complete the correct number of participants, we will over-recruit to allow for screen failures and exclusion from primary outcome analysis for natural colonisation and loss of participants due to drop out. Based on the assumption that two isolates will be tested and estimating a 20% rate of drop out/screening failure, we will recruit a maximum of 117 participants. Participants who are natural SPN carriers will be included in analysis of exploratory outcomes.

Statistical Analysis Plan

This is an open label, non-randomised, safety and dose escalation study in healthy participants. Analyses are descriptive.

Timing of Analysis

Data will be reviewed at the middle and the end of follow up for each cohort of 10 healthy participants from the dose ranging phase as soon as database completion and lock occurs for that cohort. Data reviewed after day 7 will be for targeted booster inoculations if participants have tested negative for SPN3 on both days 2 and 7. Safety and outcome results will be reported to the trial steering committee (TSC) and trial management group (TMG). Interim analysis in the dose ranging phase is to consider addition of second inoculation dose. Analysis at the end of each cohort is to consider dose escalation (or cessation). Once the dose has been selected we will complete then reproducibility study and final analysis will be done on completion of that cohort after database completion and lock.

Analysis Populations

The intention to treat population is all participants who have been enrolled and who have received at least one inoculation dose (ITT). The main analysis population for each cohort is the modified intention to treat population (mITT), consisting of all participants who have been enrolled and received at least one inoculation dose (D0) and have had at least one valid outcome assessment measure (nasal wash). The safety analysis population is the ITT population and will include all participants who have received at least one inoculation dose.

Analysis will use available data. No imputation for missing endpoints will be performed.

Covariates and Subgroups

Most analysis will evaluate outcomes overall, and then for single vs two inoculation doses in the event of a second inoculation dose. Summaries will also be stratified by time point following inoculation.

Interim Analysis and Data Monitoring

Data will be reviewed on an ongoing basis for any safety or adverse events and will be reported per protocol to the TSC and data and safety monitoring committee (DSMC). Efficacy outcomes will be evaluated after day 7 to provide information on second dose and at the cohort completion to assist with decision making for the next dose escalation in sequence. The reproducibility cohort will have a single interim look, to evaluate second inoculation dose and a final analysis at the end of cohort follow up.

Efficacy Analysis

Each dose cohort (dose selection and reproducibility) will be analysed separately. Binary endpoints will be summarised as frequency (%) with 95% confidence estimates at each time point and by participant level summary. Density will be summarised using number, mean, geometric mean, standard deviation, geometric standard deviation, minimum and maximum at each time point. 95% confidence intervals will be estimated for geometric mean estimates. Duration of colonisation (days) will be analysed as a continuous variable and summarised using mean, standard deviation, minimum and maximum in the event of no censored outcomes. In the event of censoring, product limit methods will be used to estimate endpoints. The duration will be defined as the time between inoculation and the time of the last NW

sample that is positive for colonisation. Symptom and sore through grading scales will be summarised by frequency (%). Agreement between colonisation results from microbiological culture and qPCR will be estimated using Bland-Altman plots and estimates of agreement.

The reproducibility cohort alone will use generalised linear models for single measurement outcomes (colonisation at any time vs not) under a binomial model with logit link to estimate odds ratio (95% confidence interval) for strain/procedure/one or two doses as independent variables. Censored endpoints (time to colonisation) will be analysed using product limit estimates of median (95% confidence interval for the time). Exploratory immunological outcomes will be summarised as above.

Reporting Conventions

P values > 0.0001 will be reported to 4 decimal places; p-values < 0.0001 will be reported as "< 0.0001". Distribution estimates such as mean, geometric mean, standard deviation, median and quartiles will be reported to 3 decimal places. Parameters estimates such as regression coefficients, confidence intervals and hazard ratios will be reported to three significant digits.

Safety Reporting

Adverse events (AEs) will be graded using the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Paediatric Adverse Events (20). If the severity of an AE could fall in either one of two grades, the higher of the two grades should be selected.

Symptoms experienced and attendance to hospital/GP will be asked about at each visit. Serious adverse events (SAEs) will be reported from the time of consent until completion of day 28 visit, or until completion of antibiotic treatment if participants require this.

All AEs will be recorded in the eCRF and documented in a weekly safety report. Adverse events of special interest (AESI) such as (but not limited to) headache, cough, sore throat and earache will be specifically documented and reported to the TMG, TSC and DSMC in the safety report.

All SAEs will be recorded on an SAE form and reported to the DSMC, Sponsor within 24 hours of discovery or notification of the event. All SAEs/AESIs will be followed until resolution/stabilisation or until the end of the participants last study visit. The DSMC will perform an independent review of SAEs.

Auditing

A Trial Monitoring Plan will be developed by the Sponsor and agreed by the TMG and CI based on the trial risk assessment. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted, and data are generated, documented and reported in compliance with the protocol, good clinical practice and the applicable regulatory requirements.

ETHICS AND DISSEMINATION

Research Ethics Committee Review

This protocol has been reviewed by the sponsor, funder and an external peer review process. Ethical approval has been obtained from Liverpool Central Research Ethics Committee (REC) with REC reference number 22/NW/0051. The protocol, informed consent form, participant information leaflet

(PIL) and any proposed advertising material has been approved by REC as well. For any amendment to the study, the CI, in agreement with the sponsor, will submit information to REC and other appropriate bodies. Amendments will be discussed with participants.

Consent

Potential participants will be sent a copy of the PIL (Supplemental Material 1) and invited to contact a member of the team if they would like to participate. They will then be invited to attend a presentation and to carry out a quiz to ensure they have understood the information given. If a participant has voluntarily agreed to take part in the research and the study team are satisfied that they meet the eligibility criteria, they will be invited to provide written informed consent with a delegated, trained member of staff (Supplemental Material 1). In line with recommended practice (MRC tissue and biological samples for use in research), participants will be asked to consent to gift their anonymised samples for use in future studies and shared with research collaborators and stored for any future commercial respiratory partnerships. This is outlined in the PIL and consent form.

Data Management and Participant Confidentiality

Study data will be recorded directly into REDcap, an Electronic Data Capture (EDC) system (21). Any additional information that needs recording but is not relevant for the case report form (CRF) will be recorded on a separate paper source document. The electronic CRF (eCRF) must be completed by designated and trained study personnel. Quality control will be performed on each eCRF. The processing of eCRFs will include an audit trail, to include changes made, reason for change, date of change and person making change.

Each participant will be assigned a unique, non-identifiable study number at recruitment for anonymisation. Unlinked non-identifiable clinical data will be stored and analysed at the LSTM, MSD or collaborating laboratories. Only authorised members of the clinical research team will be able to access participant personal information which is directly relevant to the study. All electronic records containing personal information will be stored in a password protected database on a password protected computer. Paper documentation containing personal information will be kept in a locked filing cabinet in a locked room. On completion of the study, the eCRF will be locked and source documents will be photocopied and archived on paper and electronically in a secure database. This data will be stored for a minimum of 25 years. We will publicise a de-identified data set to an appropriate data archive within 3 years from study completion. See Supplemental Material 2 for information on storage of biological specimens.

Dissemination Policy

The findings from this study will be disseminated amongst the scientific community. We intend to publish our findings in peer reviewed scientific journals and present data at appropriate local, national and international conferences. In addition, we will produce a report of our findings, which will be made available to all participants. Authorship of the final trial report and subsequent publications will include those who contribute to the design, delivery and analysis of the trial. Authorship will be defined on study completion in line with International Committee of Medical Journal Editors guidelines (22).

Patient and Public Involvement and Engagement

This study is run in conjunction with the EHPC studies, which have been studying pneumococcal colonisation over the last 11 years. There are numerous opportunities for public and patient involvement: newsletters are sent out to all participants to inform them of the study results and further work, previous participants assist with recruitment events and social media accounts update followers about current studies and our ongoing work. For this study, we have asked participants from previous studies to review the Participant Information Leaflet and consent form, to ensure it is clear and easy to understand.

ACKNOWLEDGEMENTS

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AUTHOR CONTRIBUTIONS

Study design set up: PH, RR, MF, CS, AHW, DMF, AMC, KL, ML, AH, TKN, SBG

Statistics: ML

Ethics application: PH, KD, MF, AMC

Study coordination: PH, CS, AHW, MF, KD, AMC, AB, JB, HF

Clinical cover including on-call responsibility: KL, RR, PH, AMC, JB, HF, AB

Writing the protocol: PH, MF, RR, CS, KL, KD, AHW, EM, BU, DMF, AMC, ML

Bacterial selection, bacterial inoculum preparation: CS, AH, TKN, DES, SBG

Manuscript writing RH, RR, MF, CS, AMC, DMF

Manuscript review PH, RR, MF, CS, AMC, DMF, SBG, KL, AHW, AH, ML, AB, HF, KD, TKN, BU, SBG, DES

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Competing interests statement

Neither the CI nor any collaborator has any direct personal involvement in organisations sponsoring or funding the research that may give rise to a possible conflict of interest.

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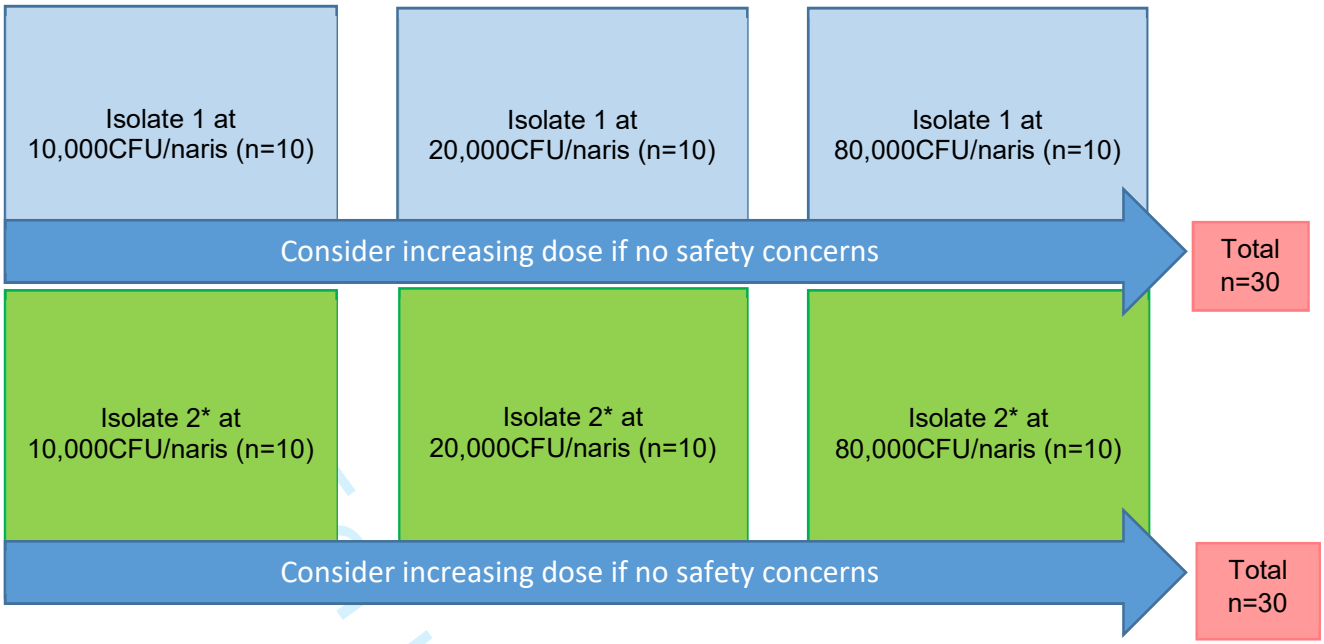
Word Count (excluding abstract, article summary, tables and references) = 4340 words

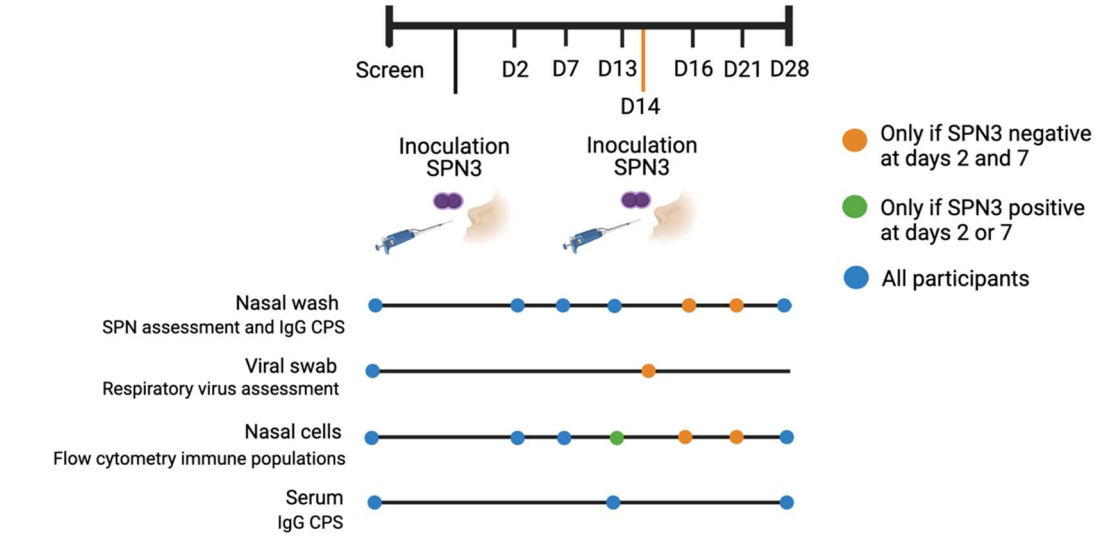
Abstract word count: 300 words



The dose may be escalated before a cohort of n=10 is inoculated, and subsequent escalated doses may be omitted if the colonisation rates are low. This is in the interest of not inoculating participants if it could be futile, and will be at the discretion of the CI

Reproducibility study with selected dose and isolate as decided by Trial Steering Committee

(n=33)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D16
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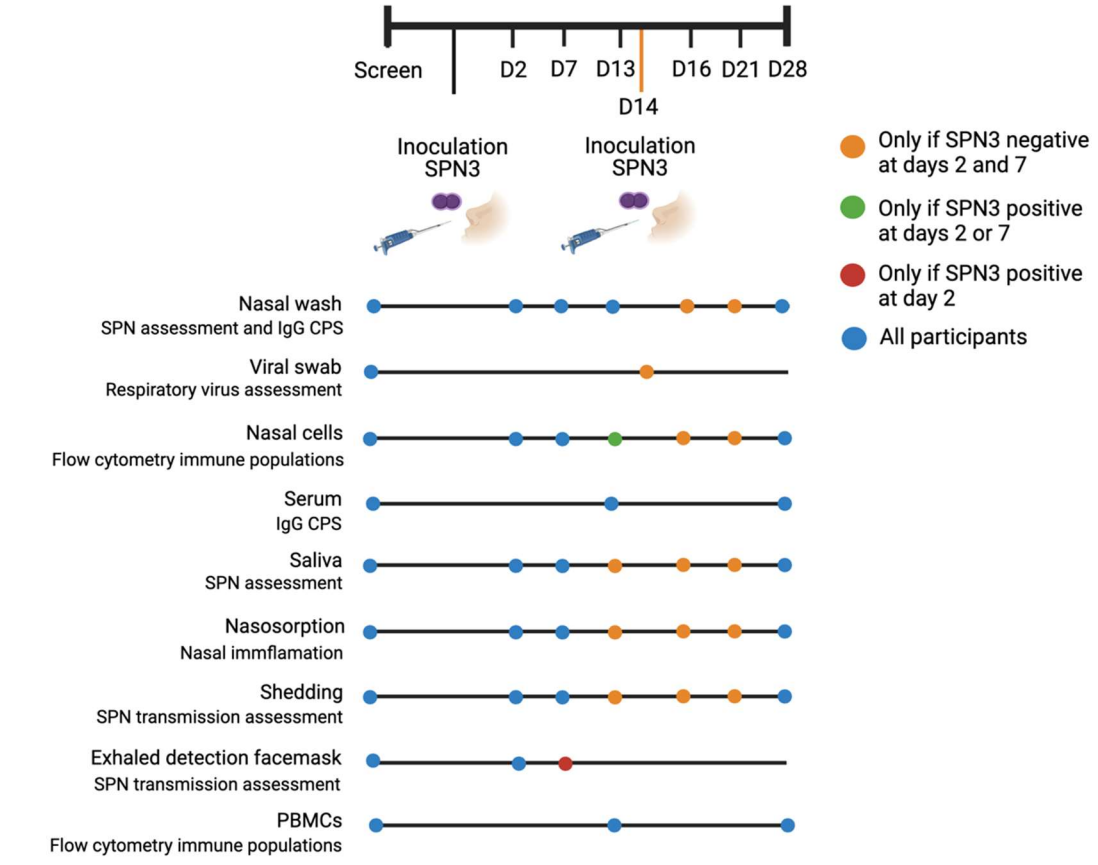
D28
Inoculation SPN3
Inoculation SPN3
Only if SPN3 negative at days 2 and 7
Only if SPN3 positive at days 2 or 7
All participants
Nasal wash
SPN assessment and IgG CPS
Viral swab
Respiratory virus assessment
Nasal cells
Flow cytometry immune populations
Serum
IgG CPS

A)


Screen
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Inoculation SPN3
Inoculation SPN3
Only if SPN3 negative at days 2 and 7
Only if SPN3 positive at days 2 or 7
Only if SPN3 positive at day 2
All participants
Nasal wash
SPN assessment and IgG CPS
Viral swab
Respiratory virus assessment
Nasal cells
Flow cytometry immune populations
Serum
IgG CPS
Saliva
SPN assessment
Nasosorption
Nasal immflamation
Shedding
SPN transmission assessment
Exhaled detection facemask
SPN transmission assessment
PBMCs
Flow cytometry immune populations

B)

ARC ACCELERATOR RESEARCH CLINIC

LSTM LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Experimental Human Pneumoccocal Challenge Model (EHPC)

Participant Information Leaflet (PIL)

Challenge 3 Study:

This information leaflet tells you how you could take part in our research. Please ask a member of the team if you have questions. You may want to talk to other people about the study: please do so. Take your time to decide if you want to be involved.

What is the purpose of the study?

We are studying a bacteria called pneumococcus which are often found in the noses of healthy adults and children without causing any symptoms or disease. However, in some people, such as older age, chronically ill adults or very young children, it is more likely to cause illness. Mild infections with pneumococcus are very common, such as ear infections in children. Less frequently, the bacteria can infect the lung (causing pneumonia), the brain (causing meningitis) or the blood (causing sepsis). These more serious illnesses are very uncommon in healthy adults. It is thought that small numbers of this bacteria in the nose ("nasal colonisation") may actually protect against pneumococcal disease such as pneumonia.

The 'Experimental Human Pneumococcal challenge' (EHPC) model is a way of putting drops of bacteria into the nose. We have studied this model of putting bacteria in the nose safely in over 1500 volunteers over the past decade with no serious side effects. We will now use a different strain of the bacteria that is commonly found in the community, called SPN3, in this model.

The aim of this study is to determine how much pneumococcus is needed to achieve nasal colonisation and how long the bacteria live in the nose for before it is cleared by natural immunity. By doing this, we will then be able to test how well future vaccines may work to prevent pneumococcal colonisation and ultimately pneumococcal infections such as pneumonia.

Do I have to take part?

No. Taking part in this study is voluntary.

Why have I been asked to take part?

We are looking for up to 117 participants aged 18-50 years old and that are fit and healthy. If we find any reason that you or your close contacts may be at higher risk of infection, then we will not ask you to take part.

The main reasons that you would not be able to take part:

- Current daily smoking (includes e-cigarettes) or significant history of smoking
- Currently involved in another study or involvement in EHPC studies in past year (3 years if involving SPN3)
- Received a pneumococcal vaccine (routine in UK if born since 2005)
- Allergy to Penicillin/ Amoxicillin
- Increased risk of infection due to chronic condition or medication
- Long term use of antibiotics
- Pregnancy or trying to conceive
- History of drug or alcohol abuse
- Directly caring for someone who has lower immune levels (patients, children under 5, the elderly) without personal protective equipment
- Overseas travel planned in follow up period

What happens if I choose to take part?

If you choose to take part in this study and the research team agrees that you are eligible, you will be asked to sign the consent form.
The study will involve 8-9 clinic visits over approximately 4-5 weeks.

What samples do you take and what are the risks?

Nasal wash: We gently squirt a little salty water into your nose. After a few seconds the water runs out into a sample bowl. This will tell us about the bacteria in your nose and your immune response.
Risk: may swallow some salty water, temporary discomfort.

Throat swab(s): We take a small cotton swab and wipe the back of your throat in a circular motion. This is used to detect bacteria and viruses in your throat.
Risk: might make you gag a little.

Nasosorption: To collect cells from your nose we place a small piece of paper into your nostril for two minutes.
Risk: Little if any discomfort

Nasal cells: We insert a very small plastic spoon (like a tooth pick) to collect cells from inside the nose. We will perform this twice on each nostril.
Risk: Temporary discomfort, eyes watering, spots of blood from the scrape.

Blood samples: We take a blood sample from a vein in your arm (using a needle). We will take up to 80mL (about the same as 8 tablespoons) during a visit. This amount of blood is safe to give, and your body will replace this blood quickly.
Risk: some people may feel faint or experience bruising.

Shedding: We use gentle methods to find out if bacteria move from the nose to the hand. For example a swab of your hand after rubbing your nose or coughing onto a plate that is used to grow bacteria.

Exhaled Detection Facemask: You wear a facemask with a special filter for 15 minutes
Risk: Can feel claustrophobic

Saliva: We will ask you to spit into a tube to provide approx 1ml.

Fig 1. Nasal Wash

The risks that you should consider *before* participation in this study are the risks associated with having blood taken, nasal sampling as listed above and inoculation with live bacteria.

Inoculation with pneumococcal bacteria: Because the bacteria are alive, there is a very small risk of infection to you or your close contacts. There is a low risk of middle ear infection and very low risk of sinusitis, pneumonia, meningitis or sepsis. The study is designed to ensure any risk is minimal and we do not expect anyone to develop an infection; we choose participants carefully and monitor them closely. We have experience of using this model safely in more than 1500 healthy participants with no serious side effects. We provide a safety pack as described above and access to the research team by phone 24/7. We give you a separate leaflet which explains the

safety precautions and what to do if you feel unwell.

What will happen at each visit?

This study involves 6-9 visits to the research clinic. Each appointment takes between 10 minutes up to a maximum of 60 minutes.

Consent

A member of the research team will discuss the study involvement with you, this may be done as a group presentation. You will then have the opportunity to ask questions and discuss the study with the researcher in private. If you choose to take part in the study, you will be asked to complete a questionnaire to demonstrate that you understand the study involvement before signing a consent form. We will inform your GP that you are taking part in the study.

Screening

This will take approximately 30 minutes. We will ask routine questions about your medical health and we will listen to your heart and lungs to make sure you are fit and well. At this visit, a number of samples will be taken which may include throat swabs (including a COVID-19 test), nasal wash, bloods, nasal scrapes, nasosorption and shedding samples.

Inoculation Visit

We use a dropper (pipette) to put a few drops of water containing a small number of pneumococcal bacteria into each nostril (inoculation). You will lie down in the clinic for 15 minutes after the procedure. Usually participants have no symptoms afterwards. There will be a doctor or nurse available by telephone 24 hours a day, 7 days a week to answer questions. We will give you a safety pack to keep with you throughout the study, this includes:

- A course of antibiotics to keep with you in case you are unwell
- A thermometer to check your temperature at home
- A safety information sheet
- A study contact card
- A symptom log

We will ask that you inform us of your temperature and symptoms daily for the next 3-5 days.

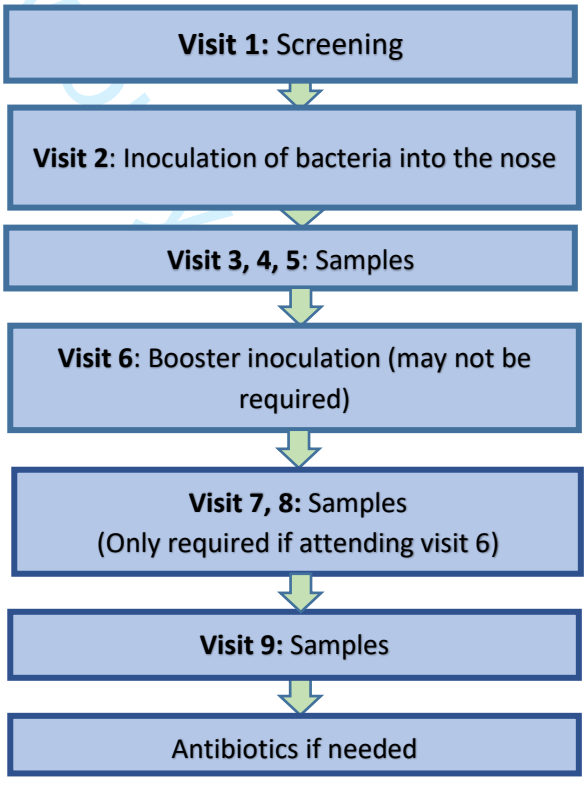
Clinic appointment visits

At each visit, a number of samples will be taken which may include throat swab(s), nasal wash, bloods, nasosorption, shedding, saliva and nasal cell samples.

End of the study

Participants that are carriers of pneumococcal bacteria at any time point, who do not go on to have negative samples, will be asked to take the antibiotics (amoxicillin 500mg 3 times per day for 5 days) from the safety pack to clear/ reduce the amount of the pneumococcus in the nose.

Appointment Flow Chart



What are the benefits of taking part?

You will be a valuable part of a research study that we hope will eventually lead to the development of new methods to prevent respiratory infections through vaccination. You will not gain any direct benefit other than a health check.

What if there is a problem?

You can contact the research team 24 hours a day by phone to answer any questions. Any medical care you need will be provided by the NHS.

What about the risk of COVID-19?

The bacteria in this study does not increase your risk of developing COVID-19 infection. To reduce the risk of COVID-19 when attending for your clinic visits the latest UK Health Security Agency guidance for 'infection prevention and control for COVID-19' will be strictly followed and you will be advised of any specific measures to be taken closer to your appointment. A swab to check for COVID-19 infection may be performed at your appointments. If you were to develop symptoms that are suggestive of COVID-19 infection (fever, cough, shortness of breath, loss of sense of smell or taste) you will be advised to follow the latest UKHSA guidance with regards to self-isolation and if required seek urgent medical attention via normal routes of healthcare.

What if I wish to complain?

If you wish to complain about any aspect of the study, you can contact the study doctor or nurse. You can also contact the sponsor by email on lstmgov@lstmed.ac.uk or telephone on 0151 702 9396. Complaining will not affect the medical care you receive now or in the future.

The study is sponsored by the Liverpool School of Tropical Medicine (LSTM) and is covered by Clinical Trial Insurance.

How much will I get paid?

The money you are paid is compensation for inconvenience, loss of income, and possible discomfort. Payments are as seen in the table below.

You will be paid between £170 and £285 for taking part in this study depending on how many visits are required and how many samples are taken on each visit

*these visits may not be required

Visit	Payment
Screen/Re-screen	£40
Inoculation	£40
Day 2	£20-£35
Day 7	£20-£35
Day 13	£20-£30
Booster inoculation Day 14*	£40*
Day 16*	£20-£25*
Day 21*	£20-£25*
Day 28	£25-£30
Minimum total:	**£170**
Maximum Total	**£285**

What if I change my mind, or want to stop?

If you do start the study, you are free to stop at any time without giving a reason. If you decide not to take part, or to withdraw from the study, this will have no effect on your future health care.

If you decide to stop, or if you lose capacity to consent during the study, we will continue to use the samples that have already been taken and information that we have already collected unless you ask us not to. You will be paid for the visits completed up to that point.

The study team may stop your involvement in the study for safety reasons.

Will my details be kept confidential?

Yes. For safety, we collect contact details and information about your medical history before you take part.

We will ask your permission to inform your GP that you are taking part in the study as this may be relevant to your medical care outside of the study. We do not expect to find anything which would affect your health care. If we do, we will let you and your GP know about it.

We will also collect information that allows us to understand more about the samples, for example, your age or sex. This will be stored on a password protected database and/or in a locked cupboard. This data may be used by LSTM researchers who need to contact you or record relevant information about the study.

Your medical notes and research data may be viewed by regulatory teams who assess the quality of the research. This is to ensure that it is conducted in accordance with Good Clinical Practice guidelines.

All data will be collected and stored at the LSTM for a minimum period of 25 years. This includes data such as your name and contact details. We use this to check if participants have already taken part in our research. We will also send newsletters and inform you about future studies.

You can find out more about how we use your information by contacting dataprotection@lstmed.ac.uk.

What will happen to my samples?

The samples taken during this study will be processed and stored in the LSTM. All samples will be anonymised at the point of sampling, the people analysing the samples and data will not have access to your personal information. The samples that you give will be gifted for future use in respiratory/infection research and stored in a research tissue bank after the study has closed. The stored samples will be analysed as and when new technology becomes available or when new scientific questions arise relating to protection and susceptibility of respiratory disease. Samples may be sent to national and international collaborating laboratories for their expertise. All identifiable information will be removed.

Experimental Human Pneumoccocal Challenge Model (EHPC)

Contact details

General questions: please contact the research team on

07740 410 290

during normal working hours.

Web site: https://www.lstmed.ac.uk/arc-volunteer-database

Emergency contact details at any time day or night: Mobile: 07912 053 981

The Chief Investigator for this study is **Dr Andrea Collins**. You may contact her at the Liverpool School of Tropical Medicine, Liverpool Life Sciences Accelerator Building, 1 Daulby Street, Liverpool, L7 8XZ, UK. Telephone: 0151 702 9439.

This research is sponsored by the Liverpool School of Tropical Medicine. It is funded by Merck. The research has been reviewed for scientific content by an external panel. The National Research Ethics Service Committee Liverpool Central has reviewed the study and given approval for it to take place.

Data protection: *If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.*

Data access: *The only people in LSTM who will have access to information that identifies you will be people who need to contact you to regarding your participation in the research or audit the data collection process. LSTM will use your name, NHS number and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from LSTM and regulatory organisations may look at your medical and research records to check the accuracy of the research study. LSTM (research site) will pass these details to LSTM (sponsor) along with the information collected from you and your medical records. The people who analyse the information will not be able to identify you and will not be able to find out your name, NHS number or contact details.*

LSTM (research site) will keep identifiable information about you from this study for a minimum of 5 years after the study has finished.

Information for research: *The information will only be used for the purpose of research and cannot be used to contact you or to affect your health care. It will not be used to make decisions about future services available to you, such as insurance.*

ARC ACCELERATOR RESEARCH CLINIC

LSTM LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Experimental Human Pneumoccocal Challenge Model (EHPC)

Consent Form

Participant Study number

Challenge 3 Study:

Please initial the box if you agree with each statement. Then, print and sign and date below.

I have read and understand the information sheet version ____**dated__/___/____** for the above study. I have been able to consider the information and ask questions. I confirm that the study procedures and information have been explained to me.	Initial
I understand that this study is voluntary and that I am free to withdraw without giving any reason without my medical care or legal rights being affected.	Initial
I understand that research notes relevant to taking part in this research and data collected may be seen by the regulatory authorities. I give permission for these people to access my records.	Initial
I agree that anonymous data and samples can be transferred outside of the UK for the purpose of the research.	Initial
I agree to my GP being informed of my participation and providing information or for the researcher to access my electronic GP summary record relevant to the study.	Initial
I understand that the samples collected will be anonymised and used and stored for the research described above, and that samples may be sent to national and international collaborating laboratories as part of the study.	Initial
I gift my samples to be used for future research in the UK and overseas. I understand that my samples are anonymised and will be transferred to a research tissue bank at the end of the study. I agree that my samples may be used in future research to investigate factors affecting infection and immunity. ……	Initial
I give permission for the study team to store my contact details in order to invite me to participate in future research	Initial
Biologically female participants of child-bearing potential: I confirm that I am not planning to conceive, and I will use effective contraception if required during the study.	Initial N/A ☐
I agree to take part in this study.	Initial

……	……	_ _/_ _ _/_ _ _ _
Name of participant (print)	Signature	Date
……	……	_ _/_ _ _/ _ _ _ _
Name of person receiving consent	Signature	Date

Experimental Human Pneumoccocal Challenge Model (EHPC)

Copies: 1 for participant, original for site file and one scanned or filed in research case notes

Supplemental Material 2. Biological Specimens

Biological samples are collected from all participants, transferred to the laboratory with an accompanying inventory form and stored, following local standard operating procedures.

The samples are “anonymised” with a participant number at the point of collection. Anonymised samples may be sent to national and international collaborators for further analysis, as detailed in the participant consent form. LSTM may store samples for up to 25yrs. After this time, the remaining samples will be transferred to a research tissue bank (LSTM).

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered,	2

		name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	1, 2, 4, 6, 7, 8, 9, 12, 15, 16
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	18
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	Supp for editors
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Supp for editors
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and	Supp for editors

		other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-6
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	N/A – no comparator used
Objectives	#7	Specific objectives or hypotheses	6-8
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can	6

		be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8-10
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-11
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	10
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	12
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	9
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6-8

Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10-11, Figures1-3
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12-13
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	10
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	N/A – no blinding used
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the	N/A – no blinding used

		sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	N/A – no blinding used
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A – no blinding used
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A – no blinding used
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be	12

		collected for participants who discontinue or deviate from intervention protocols	
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	16
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13-14
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13-14
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13-14
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is	Supp for editors

		not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	14
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	14-15
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	15
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	15
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	15
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and	15-16

		how (see Item 32)	
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	16
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	16
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	17
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	16
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	16
Dissemination policy:	#31b	Authorship eligibility guidelines and any intended use of	16

authorship		professional writers	
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	16

Appendices

Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Supp 1
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Supp 2

BMJ Open

Serotype 3 Experimental Human Pneumococcal Challenge (EHPC) study protocol; Dose ranging and reproducibility in a healthy volunteer population (Challenge 3)

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Serotype 3 Experimental Human Pneumococcal Challenge (EHPC) study protocol; Dose ranging and reproducibility in a healthy volunteer population (Challenge 3)

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Keywords: Adolescent, Adult, Healthy Volunteers, Humans, Middle Aged, Nasopharynx, Pneumococcal Pneumonia, Pneumococcal Vaccines,

Countries of recruitment: United Kingdom

Recruitment status: currently recruiting and enrolling participants

ABSTRACT

Introduction

Since the introduction of pneumococcal conjugate vaccines (PCVs), pneumococcal disease rates have declined for many vaccine-type (VT) serotypes. However, serotype 3 (SPN3) continues to cause significant disease and is identified in colonisation epidemiological studies as one of the top circulating serotypes in adults in the UK. Consequently, new vaccines that provide greater protection against SPN3 colonisation/carriage are urgently needed. The Experimental Human Pneumococcal Challenge (EHPC) model is a unique method of determining pneumococcal colonisation rates, understanding acquired immunity, and testing vaccines in a cost-effective manner. To enhance the development of effective pneumococcal vaccines against SPN3, we aim to develop a new relevant and safe SPN3 EHPC model with high attack rates which could be used to test vaccines using small sample size.

Methods and Analysis

This is a human challenge study to establish a new SPN3 EHPC model, consisting of two parts. In the dose-ranging/safety study, cohorts of 10 healthy participants will be challenged with escalating doses of SPN3. If first challenge does not lead into colonisation, participants will receive a second challenge 2-weeks after. Experimental nasopharyngeal (NP) colonisation will be determined using nasal wash sampling. Using the dose that results in ≥50% of participants being colonised, with a high safety profile, we will complete the cohort with another 33 participants to check for reproducibility of the colonisation rate. The primary outcome of this study is to determine the optimal SPN3 dose and inoculation regime to establish the highest rates of NP colonisation in healthy adults. Secondary outcomes include determining density and duration of experimental SPN3 NP colonisation and characterising mucosal and systemic immune responses to SPN3 challenge.

Ethics and Dissemination

This study is approved by the NHS Research and Ethics Committee (Reference 22/NW/0051). Findings will be published in peer-reviewed journals and reports will be made available to participants.

ISRCTN registry number: 17879306. Registered 4th April 2022. Protocol version 3.0 (7th April 2022).

ARTICLE SUMMARY

Strengths and Limitations of the Study

- The use of a novel inoculation regime of a second (targeted booster) inoculation to participants not colonised after first challenge could increase colonisation rates, (unpublished data from ISRCTN12884329); higher colonisation rates will allow smaller sample sizes in future vaccine work, reducing study costs and time, resulting in improved, more globally accessible pneumococcal vaccines faster.

- Additionally, the use of clinical globally relevant strains from the UK and Malawi not used before in EHPC studies (Serotype 3 10V CC700 and Serotype 3 LIV014-S3 CC180) will allow the independent conduct of future pneumococcal vaccine studies in various settings and potentially a future global co-challenge collaborations network.
- The evaluation of bacterial shedding of SPN3 through hand swabs, cough plates and exhaled detection facemasks is novel and could provide greater understanding of how SPN3 is spread amongst individuals, eventually leading to improved infection and prevention control in hospitalised patients.
- Follow-up beyond 14 days will allow evaluation of longer-term local immune response to SPN3 and as well as providing longer term data on colonisation rate, density and duration.
- The main limitation of this study is the similarity to the EHPC Pneumo 1 study, which used different SPN3 isolates that are proprietary to a 3rd party to establish an SPN3 model.

INTRODUCTION

Streptococcus pneumoniae (SPN) is a major cause of morbidity and mortality from a lower respiratory tract infection globally(1). Available pneumococcal conjugated vaccines (PCVs) confer protection by reducing vaccine-type (VT) SPN colonisation density and are effective against invasive pneumococcal disease (IPD) (2). After the introduction of PCV-13 into national paediatric immunisation programs, overall combined-VT IPD amongst all ages significantly reduced (3). However, surveillance data showed limited declines in serotype 3 (SPN3) IPD in all ages, despite this serotype being included in PCV-13(4). This is thought to be due to specific characteristics of the SPN3 capsule(5), which could theoretically overwhelm the protective capacity of antibodies that are produced in response to the vaccine. There could be also other evasion mechanisms apart from antibody responses that could play a role in why SPN3 is different and continues to circulate amongst vaccinated communities.

An observational study of IPD rates in England 2014-2018 showed an increase in SPN3 IPD cases, with SPN3 contributing the most to total IPD deaths(6). Furthermore, a randomised controlled trial (RCT) did not show significant difference in SPN3 colonisation post PCV-13 in infants when compared to PCV-7 (which does not include SPN3) as a control (7). Taken altogether, current data suggests that even if PCV-13 protects against SPN3 directly to some degree, it does not sufficiently produce the sustained indirect protective effects seen against other VTs. Therefore, novel vaccines providing higher levels of protection against SPN3 colonisation (and therefore disease) are needed.

The Experimental Human Pneumococcal Challenge (EHPC) model allows vaccines to be tested for their effect on experimental SPN colonisation/carriage, in a more cost-effective manner than field studies, with fewer participants and shorter follow-up (8-10). Participants are intranasally inoculated with SPN, inducing a stable colonisation episode for about 1-3 weeks, at a density typical of natural colonisation. Host samples including nasal washes, nasal cells and blood are taken to assess colonisation as well as the immune responses. The model has provided key insights into human immune mechanisms that are associated with protection and susceptibility to colonisation acquisition (11-13). The model is well developed for serotypes SPN6B and SPN15B with over 2000 challenges in over 15 independent studies, showing the model is safe and has reproducible attack rates. In recent studies the model has been used to explore bacterial shedding and the transmission potential of SPN6B, demonstrating that hands can be a vehicle for transmission of SPN6B and lead to 18% colonisation when suspensions containing the bacteria are either sniffed from the hand or inserted into the nose via a finger (14). SPN transmission has been associated with living with a larger number of people, typically in prisons or nursing homes(15), therefore evaluating methods of shedding provides invaluable insight into how this transmission occurs and can be prevented.

More recently, 96 healthy participants were challenged with three different proprietary strains of SPN3 at various doses. Colonisation rates varied from 30-70% and the model was shown to be safe and feasible (16). Interestingly, there was no increase in levels of nasal SPN3 anti-capsular antibodies in colonised participants at day-14 post-inoculation, suggesting that there could be a lack of immunogenicity with SPN3, unlike has been demonstrated in previous studies(7). Additionally, 30.2% of participants reported symptoms when questioned at routine clinic visits, of whom the majority

described a sore throat. SPN has not previously been commonly associated with pharyngitis(17) and further investigation into this is required.

To ensure the EHPC model remains at the cutting edge of pneumococcal (current and future) vaccine assessment, we are proposing here to set up an EHPC model with carefully selected non-proprietary SPN3 strains and a second (targeted booster) inoculation to achieve maximum attack rates. In this study, in addition to determining the optimal dose and isolate of SPN3 to establish highest rates of colonisation in the human nasopharynx, we intend to improve the knowledge of both mucosal and serological immune responses to SPN3 colonisation. We will investigate longer-term immune response to SPN3 colonisation (beyond 14 days) and for the first time we will use a targeted booster SPN3 inoculation to improve colonisation rates and evaluate the impact that this has on immunogenicity. We hypothesise that this could better simulate natural SPN colonisation in high transmission settings, whereby individuals are likely to be repeatedly exposed.

Additional exploratory outcomes will be assessed, including evaluating SPN3 shedding and further investigating symptoms experienced post-inoculation. The results from this study will be used to inform development of improved SPN3 vaccines and to inform design of future pneumococcal vaccine RCTs. We plan to transfer this SPN3 model to Malawi, where SPN3 is a dominant disease-causing and antimicrobial resistance-transmitting serotype. Our previous transfer of the SPN6B model has been safe and successful, revealing important differences in vaccine response in highly endemic low resource settings (18).

The primary aim of this study is to determine the optimal SPN3 dose and isolate to establish experimental nasopharyngeal colonisation in healthy adults. Success in this project will result in:

1. Expansion of the EHPC model to demonstrate safe SPN3 colonisation with new non-proprietary isolates. This will allow better understanding of SPN3 colonisation dynamics and identification of correlates of protection.
2. Determination of the optimum isolate/dose and safety of SPN3 booster inoculation, to allow future testing of vaccines in double blind randomised controlled trials with even smaller numbers of participants than usual EHPC studies (and significantly less than are required for field studies).

METHODS AND ANALYSIS

Study Overview

This protocol has been reported following the SPIRIT reporting guidelines (19).

This is a human challenge study of healthy adult participants who will be nasally inoculated with well-characterised, fully sequenced-penicillin sensitive SPN3, for the assessment of acquisition of nasal pneumococcal colonisation and immune responses. We will conduct a dose-ranging study to determine the optimum SPN3 isolate and dose for safe colonisation acquisition and confirm the dose and safety in a subsequent larger cohort in a reproducibility study. This study will run from July 2022 to October

2023 at the Accelerator Research Building, Liverpool School of Tropical Medicine (LSTM), Liverpool, UK.

Figure 1 displays the study process. In the dose ranging study, sequential cohorts of 10 healthy participants will be challenged with escalating doses of SPN3. We will start at 10,000 colony forming unit (CFU)/naris and after n=10, escalate to 20,000 CFU/naris (n=10) and then 80,000 CFU/naris (n=10) if safe to do so. The first challenge cohort at 10,000 CFU/naris will start slowly with a smaller group (n=1-6) inoculated per week, for safety, before completing the group (n=10). We may increase the dose further depending on colonisation rates after consultation with the Trial Monitoring Group (TMG). If optimum attack/ colonisation acquisition rates are achieved (≥50%) in the lower doses, the higher dose escalation groups may not be completed - this will be discussed with the trial steering committee (TSC) before a decision to omit the higher doses is made. We may escalate the dose before a cohort of n=10 is inoculated, and subsequent escalated doses may be omitted if the colonisation rates are low. This is in the interest of not inoculating participants at a dose that could be futile. This will be at the discretion of the CI.

Up to 2 isolates will be tested in this manner, Serotype 3 10V CC700 and Serotype 3 LIV014-S3 CC180. In case of not achieving the desired attack rate with any of the isolates, the data obtained will be reviewed to either test an additional isolate or complete the cohort at a lower attack rate.

Using the dose, isolate and inoculation regime that results in ≥50% colonisation acquisition/attack rate, with a high safety profile, we will complete the cohort with another 33 participants to check for reproducibility of attack rate. In previous research conducted with SPN6B, colonisation rates of 58.5% were seen with single inoculation and of those who were negative after the first inoculation, a further 41.7% then became positive after a second inoculation, resulting in a combined colonisation rate following two inoculations of 70.7%.

Study Objectives and Outcomes

Study objectives and outcomes measures are demonstrated in Table 1.

Table 1. Objectives and Outcome Measures

	Objectives	Outcome Measures
Primary	To determine the optimal SPN3 dose and isolate to establish colonisation of the nasopharynx in healthy adults using the EHPC model.	The proportion of participants with experimental SPN3 colonisation of the nasopharynx, determined by SPN3 presence in classical microbiological culture in at least one nasal wash (NW) sample, at any time point following one or two inoculations (combined and individually). This will be assessed for each isolate and dose separately.

Secondary	To ascertain the rate of experimental colonisation acquisition of SPN3 in healthy adults post challenge, by classical and molecular methods.	The rate of occurrence of SPN3 experimental colonisation of the nasopharynx, determined by SPN3 presence in classical microbiological culture and qPCR (combined and individually) from at least one NW sample at any time point following one or two inoculations (combined and individually).
	To determine the density of experimental SPN3 colonisation of the nasopharynx.	The bacterial density of experimental SPN3 colonisation of the nasopharynx in NW, at each and any time point following one or two inoculations (combined and individually), determined by classical microbiological culture and molecular methods.
	To determine the duration of experimental SPN3 colonisation of the nasopharynx.	The duration of experimental SPN3 colonisation of nasopharynx determined by the last NW sample following one or two inoculations (combined and individually) in which SPN3 is detected by classical microbiological culture or molecular methods.
Exploratory	To determine grading score of symptoms during experimental colonisation (e.g., sore throat, rhinitis, nasal congestion).	The presence of mild or moderate symptoms as recorded on a Likert scale in participants with SPN3 within the first 7 days after inoculations. Sore throat grading score will also be used if applicable.
	To characterise mucosal immune cell populations and dynamics in response to SPN3 experimental inoculation in nasal cell samples.	Cell immunophenotyping using flow cytometry methods to identify and characterise cell populations such as neutrophils, monocytes, T cells and B cells in nasal cells samples at screening and 2, 7, 13, 16, 21 and 28 days after first inoculation.
	To determine the level of mucosal and systemic SPN3 polysaccharide-specific antibodies at baseline and after SPN3 experimental inoculation.	Measurement of anti-SPN3 polysaccharide specific immunoglobulin G (IgG) levels in serum and nasal wash samples using ELISAs.
	To determine the levels of polysaccharide specific SPN3 memory B cells at baseline and after SPN3 experimental inoculation.	Quantification and characterisation of the number of SPN3- polysaccharide specific memory B cell populations in PBMC samples using flow cytometry methods at screening and 13 and 28 days after first inoculation.
	To describe nasal inflammatory kinetics induced by SPN3 experimental inoculation.	Measurement of 30 cytokines and chemokines using multiplex Luminex in nasosorption samples at screening and 2, 7, 13, 16, 21 and 28 days after first inoculation.

	To assess bacterial shedding after pneumococcal colonisation.	The rate of pneumococcal bacterial shedding as defined by swabs of hand and cough-plate based assessment post inoculation (presence and density CFU/ml) at 2, 7, 13, 16, 21 and 28 days after first inoculation. (During COVID-19 pandemic cough sampling may not be performed).
	To compare 'exhaled detection facemask' (EDF) with cough plate-based methods of assessing SPN3 bacterial shedding post-pneumococcal inoculation.	The rate of pneumococcal bacterial shedding as defined by exhaled detection facemask and cough-plate based assessment post- inoculation at 2- and 7-days post-inoculation (presence and density [CFU/ml])
	To determine the effect of natural SPN (non-SPN3) colonisation on SPN3 colonisation.	The rate of occurrence and density of SPN3 colonisation of the nasopharynx post-inoculation in natural SPN carriers, determined by SPN3 presence and density in classical microbiology culture or qPCR from at least one NW sample at any timepoint post-inoculation.
	To compare rates, density and duration of SPN3 colonisation in saliva vs nasopharyngeal samples.	The presence, density and duration of SPN3 colonisation in NW and saliva samples at any timepoint post-inoculation, identified using classical microbiology culture or qPCR

Study Participants

Inclusion/Exclusion Criteria

Healthy participants aged 18-50 (inclusive) who are fluent in English, have access to a mobile telephone and have capacity to give informed consent will be allowed to participate. This age range minimises the risk of IPD and allows comparison with our previously published EHPC work.

Tables 2 outlines the exclusion criteria for this study.

Table 2. Exclusion Criteria

Research participant	Currently involved in another study unless observational or non-interventional, excluding the EHPC bronchoscopy study and COVID-19 observation and interventional trials
	Participant in any previous EHPC trial in past 1 year
	Previous EHPC trial inoculated with SPN3 in last 3 years
Vaccination	Previous pneumococcal vaccination PPV23 or PCV13 or PCV10.
Allergy	Allergy to penicillin

Comorbidities	Chronic respiratory, cardiac, kidney, liver or neurological disease
	Connective tissue disease
	Diabetes
	Immunosuppressive disease
	Recurrent otitis media
	Asplenia or spleen dysfunction
	Cochlear Implants
	Major cerebrospinal fluid leak
	Uncontrolled medical/surgical conditions (at discretion of study doctor)
	Major pneumococcal illness requiring hospitalisation within the last 10 years
	Other conditions considered by clinical team as a concern for safety/integrity of the study
	Significant mental health problems (uncontrolled or previous admission to psychiatric unit)
Medications	Immunosuppressive medication
	Long-term antibiotic use or use of antibiotics in 28 days prior to inoculation
Direct caring role or close contact with individuals at higher risk of infection (during the EHPC period) unless wearing personal protective equipment	Children aged under 5
	Chronic ill health or immunosuppressed adults
	People that are part of extremely vulnerable group as defined by Public Health England
Smoking/drug/alcohol use	Current or ex-smoker (daily cigarettes/e-cigarettes/smoking of recreational drugs) in the last 6 months. Participants who smoke <5 cigarettes per week may be included
	Previous significant smoking history (>20 pack years)
	History or current drug or alcohol abuse (frequently drinking alcohol): men and women should not regularly drink >3 units/day and >2 units/day respectively) at discretion of the clinician
Biologically female participants of child-bearing potential who are currently pregnant/lactating, intending on becoming pregnant or not on effective birth control	
Overseas travel (involving air travel) planned in follow up period of study visits	
Participants who meet following criteria at time of screening: • Unexplained/concerning findings on history/examination • Haemoglobin <90g/dL • White cell count (WCC) <1.5 x10^9/l or >12 x10^9/l	

- Platelets <75 x10^9/l
- Oxygen saturations <94%

Additionally, we will employ temporary exclusion criteria, including:

- COVID-19 symptoms or confirmed current COVID-19 infection.
- Current/acute illness within 14 days prior to inoculation if COVID-19 negative.
- Positive COVID-19 swab within 10 days of inoculation. Participants will require negative lateral flow test prior to inoculation.
- COVID-19 vaccination 21 days prior to inoculation.
- Natural SPN3 colonisation identified in baseline nasal wash.

Participants who have been temporarily excluded at screening may be re-screened at a later date to assess their eligibility at this time for inclusion into the study.

Participant recruitment

Participants will be recruited from the general public, including through public engagement events, social media, generic research communication mailing lists, large local employers and local universities.

Participant Timeline

For both parts of the study, participants will attend an identical visit schedule, however the samples taken at these visits will differ (Figure 2). Participants will attend a screening visit, inoculation visit (day 0) and then follow-up visits at days 2, 7, 13 and 28. The screening visit should occur 5 days prior to inoculation but a window of -7/+4 days will be used. Figure 2 outlines the samples that are required at each visit.

All will first attend a screening visit to confirm eligibility through medical history, clinical examination and acquisition of samples including a full blood count and nasal wash sampling. Baseline nasal washes will be evaluated for natural colonisation with SPN (and serotype if present) through classical microbiological culture and molecular methods.

All participants will then attend an inoculation visit (day 0), where fluid containing SPN3 will be instilled into their nose. At this visit, they will be given a safety pack containing a thermometer, safety information leaflet and a 5-day course of amoxicillin. They will be instructed to contact the research team daily for 3-5 days with their temperature recording and any symptoms. Participants who report symptoms consistent with pneumococcal disease will be reviewed in person by a clinician and may be instructed to take their antibiotic course. Participants have 24-hour access to research clinicians as well as access to hospital facilities and prompt treatment if required.

In the dose-ranging study, a second targeted booster inoculation will be given to participants on day 14 if they have tested negative for SPN3 on day 2 and 7 samples. In the reproducibility study, it will be at the discretion of the chief investigator (CI) to decide if targeted booster inoculation should be applied, based on results of the dose-ranging study. If a second inoculation is included in the reproducibility

study, it will be only given at day 14 to those participants who are negative for SPN3 at days 2 and 7 (these participants will be asked to attend additional follow-up visits at days 16 and 21 for further sampling).

During the dose-ranging phase, nasal wash samples pre- and post-inoculation will be collected to assess colonisation acquisition and density, by classical microbiological culture and molecular methods. Additionally, serum samples will be taken at baseline, 13 and 28-days after inoculation, to measure levels of anti-capsule polysaccharide immunoglobulin G (IgG). Nasal cell samples will be taken to characterise cellular populations and dynamics by flow cytometry.

During reproducibility phase, nasal wash and nasal cell samples will be collected as in the dose-ranging study. A viral swab will be collected pre-inoculation to test for viral co-infection. Nasal filters (nasosorption) will be collected pre- and post-inoculation to assess mucosal inflammation using 30-plex Luminex. Saliva samples will be taken pre- and post-inoculation to compare rates, density and duration of SPN3 colonisation in saliva and nasopharyngeal samples**.** Peripheral blood mononuclear cells (PBMCs) will be obtained at baseline, at 13-days and at 28-days post-inoculation. PBMCs will be used to characterise memory B cell and other immune cell populations using flow-cytometry based methods. Serum samples will be taken at baseline, 13 and 28 days after inoculation to measure levels of anti-capsule polysaccharide IgG. Exhaled detection facemasks (EDF) will be collected at day 2, to allow comparison against other shedding samples for detection of SPN3. A subgroup of participants who have demonstrated colonisation with SPN3 at day 2 will undergo EDF sampling at day 7.

At the end of the study, study participants who have been positive for SPN3 colonisation at any time point, and who have not subsequently had two consecutive negative nasal wash samples, will be asked to take oral amoxicillin 500mg three times daily for 5 days with the aim to clear / assist with clearing of colonisation.

Participant Retention

We will use an online booking system for appointments, to ensure that reminders are sent to participants for each appointment. There are windows of 2 days around all appointments to allow participants to move their appointments within window, if needed. Participants are remunerated on study completion.

Data Collection Methods

Samples will only be collected by staff members who are trained and delegated to do so. Table 3 describes the data collection methods.

Table 3. Data Collection Methods

Determination of colonisation	Colonisation will be defined as result of nasal washes taken at 2, 7, 13, 16, 21 and 28-days post-inoculation. Nasal washes will be performed using the Naclerio method (14), which is a validated technique to collect nasal bacterial specimens. Nasal washes will be plated onto culture media. Colonies will be confirmed as SPN3 using classical microbiological techniques. Results from the cultured nasal

	wash will also be confirmed using Polymerase Chain Reaction (PCR) based methods.
Molecular methods of determination of colonisation	DNA will be extracted from bacterial pellet post nasal wash sample centrifugation. SPN3 detection will be done by multiplex qPCR. This technique will enable us to detect individuals who are potential carriers with very low bacterial density. This multiplex qPCR is well validated in our laboratory (16).
Viral detection and quantification	Viral multiplex qPCR for detection and quantification will be performed on DNA and RNA of stored throat swab and/or nasal wash to detect all common respiratory viruses.
Mucosal and systemic immune responses	Serotype-specific responses and their association with both acquisition and clearance of colonisation (density and duration) will be measured. We will compare antibody levels and function between those colonised and those protected against colonisation. Levels of immunoglobulin to the capsular polysaccharides SPN3 in serum and nasal washes before and after inoculation will be determined. Levels of SPN3-specific memory B cells will be assessed using PBMCs collected pre and post inoculation. Flow cytometry will be used to examine the induction of antigen-specific cellular responses in blood including B cell and T cells. Mucosal cellular responses will also be measured by flow cytometry on nasal cell samples. Additionally, the mucosal inflammatory response associated with inoculation will be evaluated using 30-plex Luminex method to detect cytokines and chemokines in nasal filters

Sample Size Calculation

We have adopted a step-wise approach to escalating the inoculation dose. The protocol is designed to: a) minimise the possibility that we try repeatedly to attain colonisation at a dose in which it is unlikely to happen; b) maximise safety by inoculating small groups before continuing onto larger groups (in which we will have the statistical power to give reasonable precision of our estimate of colonisation rates).

Based on previous experience in studies of SPN6B and SPN3, we expect that with a colonisation rate of 45%, with 95% confidence level, and a margin of error of 15%, our study will be complete 43 participants with a single inoculation dose. Depending on colonisation rates at different inoculum doses of the SPN3 isolate, we need a minimum of 43 and a maximum of 93 participants to complete the study. This allows for up to two isolates to be taken through to the highest dose of 80,000 CFU/100µL. For example, if two isolates were tested at each dose in 10 participants in the dose ranging study, this would equate to 60 participants. A further 33 participants would then take part in the reproducibility study, equating to a total of 93 participants. To ensure that we complete the correct number of participants, we will over-recruit to allow for screen failures and exclusion from primary outcome analysis for natural colonisation and loss of participants due to drop out. Based on the assumption that two isolates will be tested and estimating a 20% rate of drop out/screening failure, we will recruit a maximum of 117

participants. Participants who are natural SPN carriers will be included in analysis of exploratory outcomes.

Statistical Analysis Plan

This is an open label, non-randomised, safety and dose escalation study in healthy participants. Analyses are descriptive.

Timing of Analysis

Data will be reviewed at the middle and the end of follow up for each cohort of 10 healthy participants from the dose ranging phase as soon as database completion and lock occurs for that cohort. Data reviewed after day 7 will be for targeted booster inoculations if participants have tested negative for SPN3 on both days 2 and 7. Safety and outcome results will be reported to the trial steering committee (TSC) and trial management group (TMG). Interim analysis in the dose ranging phase is to consider addition of second inoculation dose. Analysis at the end of each cohort is to consider dose escalation (or cessation). Once the dose has been selected we will complete then reproducibility study and final analysis will be done on completion of that cohort after database completion and lock.

Analysis Populations

The intention to treat population is all participants who have been enrolled and who have received at least one inoculation dose (ITT). The main analysis population for each cohort is the modified intention to treat population (mITT), consisting of all participants who have been enrolled and received at least one inoculation dose (D0) and have had at least one valid outcome assessment measure (nasal wash). The safety analysis population is the ITT population and will include all participants who have received at least one inoculation dose.

Analysis will use available data. No imputation for missing endpoints will be performed.

Covariates and Subgroups

If targeted booster inoculations are given, the numbers and results of these will be described, including a summary of colonisation after the second dose. Depending on the number receiving second doses, and on the endpoint being analyses, the results after the second dose will either be included as a 'new' inoculation event, potentially requiring adjustment for repeated measures, or will be excluded. Immunological data will be described, using appropriate estimators for the characteristics of the variable (arithmetic or multiplicative means, for example) and appropriate transformations as required. Where appropriate, and in particular for the reproducibility cohort, regression models will be used to estimate adjusted associations between strain and number of doses with colonisation as an outcome, and with immunological response as an outcome.

Interim Analysis and Data Monitoring

Data will be reviewed on an ongoing basis for any safety or adverse events and will be reported per protocol to the TSC and data and safety monitoring committee (DSMC). Efficacy outcomes will be evaluated after day 7 to provide information on second dose and at the cohort completion to assist with decision making for the next dose escalation in sequence. The reproducibility cohort will have a single interim look, to evaluate second inoculation dose and a final analysis at the end of cohort follow up.

Efficacy Analysis

Each dose cohort (dose selection and reproducibility) will be analysed separately. Binary endpoints will be summarised as frequency (%) with 95% confidence estimates at each time point and by participant level summary. Density will be summarised using number, mean, geometric mean, standard deviation, geometric standard deviation, minimum and maximum at each time point. 95% confidence intervals will be estimated for geometric mean estimates. Duration of colonisation (days) will be analysed as a continuous variable and summarised using mean, standard deviation, minimum and maximum in the event of no censored outcomes. In the event of censoring, product limit methods will be used to estimate endpoints. The duration will be defined as the time between inoculation and the time of the last NW sample that is positive for colonisation. Symptom and sore through grading scales will be summarised by frequency (%). Agreement between colonisation results from microbiological culture and qPCR will be estimated using Bland-Altman plots and estimates of agreement.

The reproducibility cohort alone will use generalised linear models for single measurement outcomes (colonisation at any time vs not) under a binomial model with logit link to estimate odds ratio (95% confidence interval) for strain/procedure/one or two doses as independent variables. Censored endpoints (time to colonisation) will be analysed using product limit estimates of median (95% confidence interval for the time). Exploratory immunological outcomes will be summarised as above.

Reporting Conventions

P values > 0.0001 will be reported to 4 decimal places; p-values < 0.0001 will be reported as "< 0.0001". Distribution estimates such as mean, geometric mean, standard deviation, median and quartiles will be reported to 3 decimal places. Parameters estimates such as regression coefficients, confidence intervals and hazard ratios will be reported to three significant digits.

Safety Reporting

Adverse events (AEs) will be graded using the Division of AIDS (DAIDS) Table for Grading the Severity of Adult and Paediatric Adverse Events (20). If the severity of an AE could fall in either one of two grades, the higher of the two grades should be selected.

Symptoms experienced and attendance to hospital/GP will be asked about at each visit. Serious adverse events (SAEs) will be reported from the time of consent until completion of day 28 visit, or until completion of antibiotic treatment if participants require this.

All AEs will be recorded in the eCRF and documented in a weekly safety report. Adverse events of special interest (AESI) such as (but not limited to) headache, cough, sore throat and earache will be specifically documented and reported to the TMG, TSC and DSMC in the safety report.

All SAEs will be recorded on an SAE form and reported to the DSMC, Sponsor within 24 hours of discovery or notification of the event. All SAEs/AESIs will be followed until resolution/stabilisation or until the end of the participants last study visit. The DSMC will perform an independent review of SAEs.

Auditing

A Trial Monitoring Plan will be developed by the Sponsor and agreed by the TMG and CI based on the trial risk assessment. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted, and data are generated, documented and reported in compliance with the protocol, good clinical practice and the applicable regulatory requirements.

ETHICS AND DISSEMINATION

Research Ethics Committee Review

This protocol has been reviewed by the sponsor, funder and an external peer review process. Ethical approval has been obtained from Liverpool Central Research Ethics Committee (REC) with REC reference number 22/NW/0051. The protocol, informed consent form, participant information leaflet (PIL) and any proposed advertising material has been approved by REC as well. For any amendment to the study, the CI, in agreement with the sponsor, will submit information to REC and other appropriate bodies. Amendments will be discussed with participants.

Consent

Potential participants will be sent a copy of the PIL (Supplemental Material 1) and invited to contact a member of the team if they would like to participate. They will then be invited to attend a presentation and to carry out a quiz to ensure they have understood the information given. If a participant has voluntarily agreed to take part in the research and the study team are satisfied that they meet the eligibility criteria, they will be invited to provide written informed consent with a delegated, trained member of staff (Supplemental Material 1). In line with recommended practice (MRC tissue and biological samples for use in research), participants will be asked to consent to gift their anonymised samples for use in future studies and shared with research collaborators and stored for any future commercial respiratory partnerships. This is outlined in the PIL and consent form.

Data Management and Participant Confidentiality

Study data will be recorded directly into REDcap, an Electronic Data Capture (EDC) system (21). Any additional information that needs recording but is not relevant for the case report form (CRF) will be recorded on a separate paper source document. The electronic CRF (eCRF) must be completed by designated and trained study personnel. Quality control will be performed on each eCRF. The processing of eCRFs will include an audit trail, to include changes made, reason for change, date of change and person making change.

Each participant will be assigned a unique, non-identifiable study number at recruitment for anonymisation. Unlinked non-identifiable clinical data will be stored and analysed at the LSTM, MSD or collaborating laboratories. Only authorised members of the clinical research team will be able to access participant personal information which is directly relevant to the study. All electronic records containing personal information will be stored in a password protected database on a password protected computer. Paper documentation containing personal information will be kept in a locked filing cabinet in a locked room. On completion of the study, the eCRF will be locked and source documents will be

photocopied and archived on paper and electronically in a secure database. This data will be stored for a minimum of 25 years. See Supplemental Material 2 for information on storage of biological specimens.

Dissemination Policy

The findings from this study will be disseminated amongst the scientific community. We intend to publish our findings in peer reviewed scientific journals and present data at appropriate local, national and international conferences. In addition, we will produce a report of our findings, which will be made available to all participants. Authorship of the final trial report and subsequent publications will include those who contribute to the design, delivery and analysis of the trial. Authorship will be defined on study completion in line with International Committee of Medical Journal Editors guidelines (22).

Regarding data sharing, we will hold exclusivity of participant data for 2 years, until all analysis and publications are complete. Deidentified individual participant data that underlie the results reported at the end of the trial will then be made available in an open format with accompanying metadata to the LSTM archive team, who can make it available on request. Researchers who provide a methodologically sound proposal will be able to request access, to achieve aims in the agreed proposal. Proposals may be submitted up to 5 years following publication of the results of this protocol. After 5 years, the data will be available in LSTM archives but without investigator support.

Patient and Public Involvement and Engagement

This study is run in conjunction with the EHPC studies, which have been studying pneumococcal colonisation over the last 11 years. There are numerous opportunities for public and patient involvement: newsletters are sent out to all participants to inform them of the study results and further work, previous participants assist with recruitment events and social media accounts update followers about current studies and our ongoing work. For this study, we have asked participants from previous studies to review the Participant Information Leaflet and consent form, to ensure it is clear and easy to understand.

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AUTHOR CONTRIBUTIONS

Study design set up: PH, RR, MF, CS, AHW, DMF, AMC, KL, ML, AH, TKN, SBG

Statistics: ML

Ethics application: PH, KD, MF, AMC

Study coordination: PH, CS, AHW, MF, KD, AMC, AB, JB, HF

Clinical cover including on-call responsibility: KL, RR, PH, AMC, JB, HF, AB

Writing the protocol: PH, MF, RR, CS, KL, KD, AHW, EM, BU, DMF, AMC, ML

Bacterial selection, bacterial inoculum preparation: CS, AH, TKN, DES, SBG

Manuscript writing RH, RR, MF, CS, AMC, DMF

Manuscript review PH, RR, MF, CS, AMC, DMF, SBG, KL, AHW, AH, ML, AB, HF, KD, TKN, BU, SBG, DES

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Competing interests statement

Neither the CI nor any collaborator has any direct personal involvement in organisations sponsoring or funding the research that may give rise to a possible conflict of interest.

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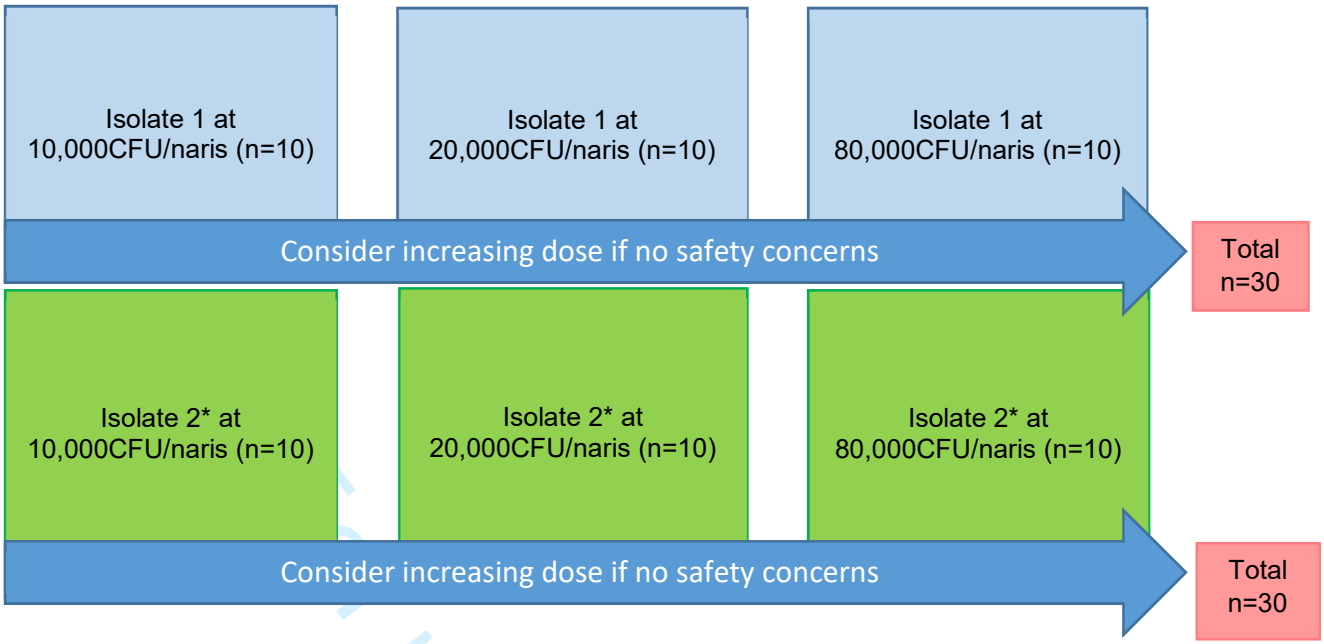
Abstract word count: 300 words

Figure Legend.

Figure 1. Trial Flow Chart

*Isolate 2 may not be tested at the discretion of the Trial Steering Committee

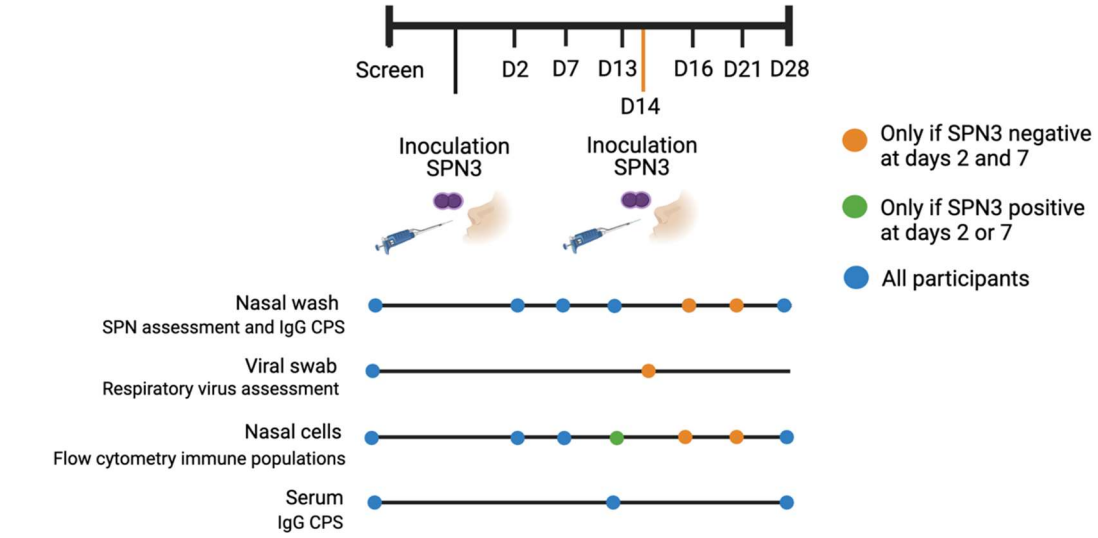
Figure 2. A) Dose-Ranging study sampling schedule. B) Reproducibility study sampling schedule. SPN Streptococcus pneumoniae, SPN3 Streptococcus pneumoniae serotype 3, CPS capsular polysaccharide, IgG Immunoglobulin G. Created with Biorender.com



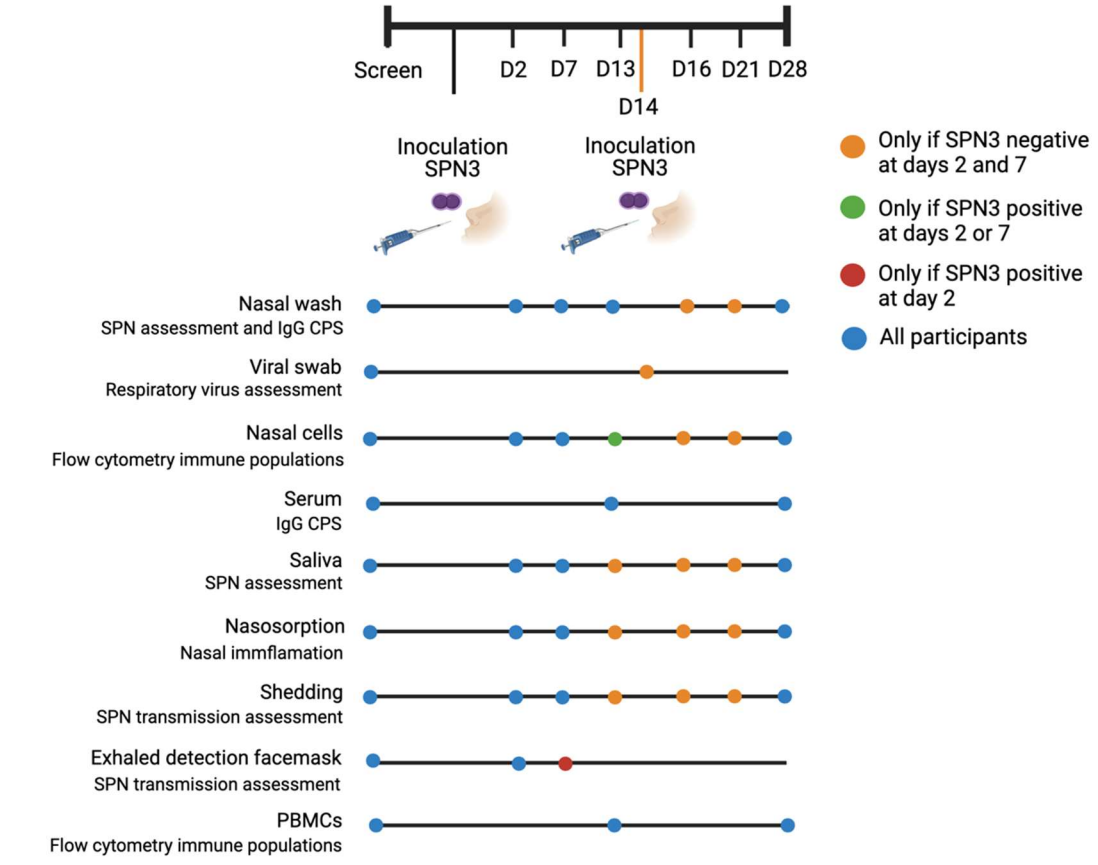
The dose may be escalated before a cohort of n=10 is inoculated, and subsequent escalated doses may be omitted if the colonisation rates are low. This is in the interest of not inoculating participants if it could be futile, and will be at the discretion of the CI

Reproducibility study with selected dose and isolate as decided by Trial Steering Committee

(n=33)


Screen
D2
D7
D13
D14
D16
D21
D28
Inoculation SPN3
Inoculation SPN3
Only if SPN3 negative at days 2 and 7
Only if SPN3 positive at days 2 or 7
All participants
Nasal wash
SPN assessment and IgG CPS
Viral swab
Respiratory virus assessment
Nasal cells
Flow cytometry immune populations
Serum
IgG CPS

A)


Screen
D2
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Inoculation SPN3
Inoculation SPN3
Only if SPN3 negative at days 2 and 7
Only if SPN3 positive at days 2 or 7
Only if SPN3 positive at day 2
All participants
Nasal wash
SPN assessment and IgG CPS
Viral swab
Respiratory virus assessment
Nasal cells
Flow cytometry immune populations
Serum
IgG CPS
Saliva
SPN assessment
Nasosorption
Nasal immflamation
Shedding
SPN transmission assessment
Exhaled detection facemask
SPN transmission assessment
PBMCs
Flow cytometry immune populations

B)

ARC ACCELERATOR RESEARCH CLINIC

LSTM LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Experimental Human Pneumoccocal Challenge Model (EHPC)

Participant Information Leaflet (PIL)

Challenge 3 Study:

This information leaflet tells you how you could take part in our research. Please ask a member of the team if you have questions. You may want to talk to other people about the study: please do so. Take your time to decide if you want to be involved.

What is the purpose of the study?

We are studying a bacteria called pneumococcus which are often found in the noses of healthy adults and children without causing any symptoms or disease. However, in some people, such as older age, chronically ill adults or very young children, it is more likely to cause illness. Mild infections with pneumococcus are very common, such as ear infections in children. Less frequently, the bacteria can infect the lung (causing pneumonia), the brain (causing meningitis) or the blood (causing sepsis). These more serious illnesses are very uncommon in healthy adults. It is thought that small numbers of this bacteria in the nose ("nasal colonisation") may actually protect against pneumococcal disease such as pneumonia.

The 'Experimental Human Pneumococcal challenge' (EHPC) model is a way of putting drops of bacteria into the nose. We have studied this model of putting bacteria in the nose safely in over 1500 volunteers over the past decade with no serious side effects. We will now use a different strain of the bacteria that is commonly found in the community, called SPN3, in this model.

The aim of this study is to determine how much pneumococcus is needed to achieve nasal colonisation and how long the bacteria live in the nose for before it is cleared by natural immunity. By doing this, we will then be able to test how well future vaccines may work to prevent pneumococcal colonisation and ultimately pneumococcal infections such as pneumonia.

Do I have to take part?

No. Taking part in this study is voluntary.

Why have I been asked to take part?

We are looking for up to 117 participants aged 18-50 years old and that are fit and healthy. If we find any reason that you or your close contacts may be at higher risk of infection, then we will not ask you to take part.

The main reasons that you would not be able to take part:

- Current daily smoking (includes e-cigarettes) or significant history of smoking
- Currently involved in another study or involvement in EHPC studies in past year (3 years if involving SPN3)
- Received a pneumococcal vaccine (routine in UK if born since 2005)
- Allergy to Penicillin/ Amoxicillin
- Increased risk of infection due to chronic condition or medication
- Long term use of antibiotics
- Pregnancy or trying to conceive
- History of drug or alcohol abuse
- Directly caring for someone who has lower immune levels (patients, children under 5, the elderly) without personal protective equipment
- Overseas travel planned in follow up period

What happens if I choose to take part?

If you choose to take part in this study and the research team agrees that you are eligible, you will be asked to sign the consent form.
The study will involve 8-9 clinic visits over approximately 4-5 weeks.

What samples do you take and what are the risks?

Nasal wash: We gently squirt a little salty water into your nose. After a few seconds the water runs out into a sample bowl. This will tell us about the bacteria in your nose and your immune response.
Risk: may swallow some salty water, temporary discomfort.

Throat swab(s): We take a small cotton swab and wipe the back of your throat in a circular motion. This is used to detect bacteria and viruses in your throat.
Risk: might make you gag a little.

Nasosorption: To collect cells from your nose we place a small piece of paper into your nostril for two minutes.
Risk: Little if any discomfort

Nasal cells: We insert a very small plastic spoon (like a tooth pick) to collect cells from inside the nose. We will perform this twice on each nostril.
Risk: Temporary discomfort, eyes watering, spots of blood from the scrape.

Blood samples: We take a blood sample from a vein in your arm (using a needle). We will take up to 80mL (about the same as 8 tablespoons) during a visit. This amount of blood is safe to give, and your body will replace this blood quickly.
Risk: some people may feel faint or experience bruising.

Shedding: We use gentle methods to find out if bacteria move from the nose to the hand. For example a swab of your hand after rubbing your nose or coughing onto a plate that is used to grow bacteria.

Exhaled Detection Facemask: You wear a facemask with a special filter for 15 minutes
Risk: Can feel claustrophobic

Saliva: We will ask you to spit into a tube to provide approx 1ml.

Fig 1. Nasal Wash

The risks that you should consider *before* participation in this study are the risks associated with having blood taken, nasal sampling as listed above and inoculation with live bacteria.

Inoculation with pneumococcal bacteria: Because the bacteria are alive, there is a very small risk of infection to you or your close contacts. There is a low risk of middle ear infection and very low risk of sinusitis, pneumonia, meningitis or sepsis. The study is designed to ensure any risk is minimal and we do not expect anyone to develop an infection; we choose participants carefully and monitor them closely. We have experience of using this model safely in more than 1500 healthy participants with no serious side effects. We provide a safety pack as described above and access to the research team by phone 24/7. We give you a separate leaflet which explains the

safety precautions and what to do if you feel unwell.

What will happen at each visit?

This study involves 6-9 visits to the research clinic. Each appointment takes between 10 minutes up to a maximum of 60 minutes.

Consent

A member of the research team will discuss the study involvement with you, this may be done as a group presentation. You will then have the opportunity to ask questions and discuss the study with the researcher in private. If you choose to take part in the study, you will be asked to complete a questionnaire to demonstrate that you understand the study involvement before signing a consent form. We will inform your GP that you are taking part in the study.

Screening

This will take approximately 30 minutes. We will ask routine questions about your medical health and we will listen to your heart and lungs to make sure you are fit and well. At this visit, a number of samples will be taken which may include throat swabs (including a COVID-19 test), nasal wash, bloods, nasal scrapes, nasosorption and shedding samples.

Inoculation Visit

We use a dropper (pipette) to put a few drops of water containing a small number of pneumococcal bacteria into each nostril (inoculation). You will lie down in the clinic for 15 minutes after the procedure. Usually participants have no symptoms afterwards. There will be a doctor or nurse available by telephone 24 hours a day, 7 days a week to answer questions. We will give you a safety pack to keep with you throughout the study, this includes:

- A course of antibiotics to keep with you in case you are unwell
- A thermometer to check your temperature at home
- A safety information sheet
- A study contact card
- A symptom log

We will ask that you inform us of your temperature and symptoms daily for the next 3-5 days.

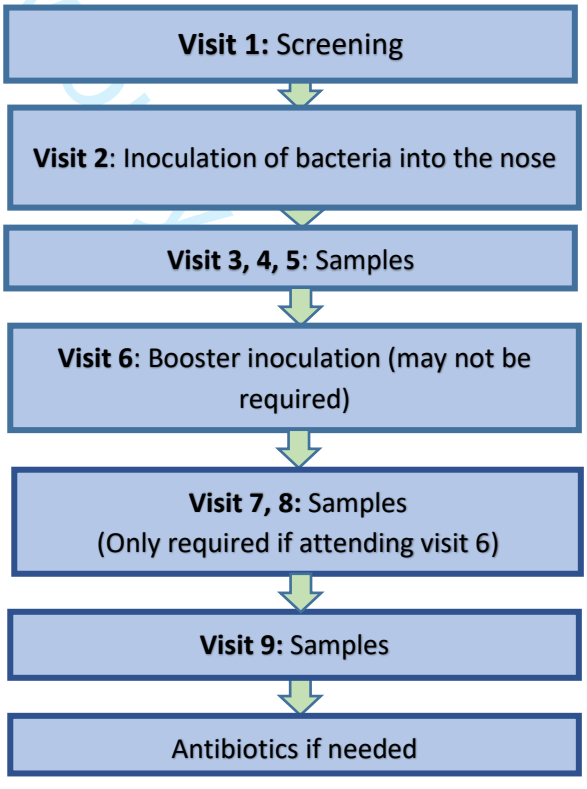
Clinic appointment visits

At each visit, a number of samples will be taken which may include throat swab(s), nasal wash, bloods, nasosorption, shedding, saliva and nasal cell samples.

End of the study

Participants that are carriers of pneumococcal bacteria at any time point, who do not go on to have negative samples, will be asked to take the antibiotics (amoxicillin 500mg 3 times per day for 5 days) from the safety pack to clear/ reduce the amount of the pneumococcus in the nose.

Appointment Flow Chart



What are the benefits of taking part?

You will be a valuable part of a research study that we hope will eventually lead to the development of new methods to prevent respiratory infections through vaccination. You will not gain any direct benefit other than a health check.

What if there is a problem?

You can contact the research team 24 hours a day by phone to answer any questions. Any medical care you need will be provided by the NHS.

What about the risk of COVID-19?

The bacteria in this study does not increase your risk of developing COVID-19 infection. To reduce the risk of COVID-19 when attending for your clinic visits the latest UK Health Security Agency guidance for 'infection prevention and control for COVID-19' will be strictly followed and you will be advised of any specific measures to be taken closer to your appointment. A swab to check for COVID-19 infection may be performed at your appointments. If you were to develop symptoms that are suggestive of COVID-19 infection (fever, cough, shortness of breath, loss of sense of smell or taste) you will be advised to follow the latest UKHSA guidance with regards to self-isolation and if required seek urgent medical attention via normal routes of healthcare.

What if I wish to complain?

If you wish to complain about any aspect of the study, you can contact the study doctor or nurse. You can also contact the sponsor by email on lstmgov@lstmed.ac.uk or telephone on 0151 702 9396. Complaining will not affect the medical care you receive now or in the future.

The study is sponsored by the Liverpool School of Tropical Medicine (LSTM) and is covered by Clinical Trial Insurance.

How much will I get paid?

The money you are paid is compensation for inconvenience, loss of income, and possible discomfort. Payments are as seen in the table below.

You will be paid between £170 and £285 for taking part in this study depending on how many visits are required and how many samples are taken on each visit

*these visits may not be required

Visit	Payment
Screen/Re-screen	£40
Inoculation	£40
Day 2	£20-£35
Day 7	£20-£35
Day 13	£20-£30
Booster inoculation Day 14*	£40*
Day 16*	£20-£25*
Day 21*	£20-£25*
Day 28	£25-£30
Minimum total:	**£170**
Maximum Total	**£285**

What if I change my mind, or want to stop?

If you do start the study, you are free to stop at any time without giving a reason. If you decide not to take part, or to withdraw from the study, this will have no effect on your future health care.

If you decide to stop, or if you lose capacity to consent during the study, we will continue to use the samples that have already been taken and information that we have already collected unless you ask us not to. You will be paid for the visits completed up to that point.

The study team may stop your involvement in the study for safety reasons.

Will my details be kept confidential?

Yes. For safety, we collect contact details and information about your medical history before you take part.

We will ask your permission to inform your GP that you are taking part in the study as this may be relevant to your medical care outside of the study. We do not expect to find anything which would affect your health care. If we do, we will let you and your GP know about it.

We will also collect information that allows us to understand more about the samples, for example, your age or sex. This will be stored on a password protected database and/or in a locked cupboard. This data may be used by LSTM researchers who need to contact you or record relevant information about the study.

Your medical notes and research data may be viewed by regulatory teams who assess the quality of the research. This is to ensure that it is conducted in accordance with Good Clinical Practice guidelines.

All data will be collected and stored at the LSTM for a minimum period of 25 years. This includes data such as your name and contact details. We use this to check if participants have already taken part in our research. We will also send newsletters and inform you about future studies.

You can find out more about how we use your information by contacting dataprotection@lstmed.ac.uk.

What will happen to my samples?

The samples taken during this study will be processed and stored in the LSTM. All samples will be anonymised at the point of sampling, the people analysing the samples and data will not have access to your personal information. The samples that you give will be gifted for future use in respiratory/infection research and stored in a research tissue bank after the study has closed. The stored samples will be analysed as and when new technology becomes available or when new scientific questions arise relating to protection and susceptibility of respiratory disease. Samples may be sent to national and international collaborating laboratories for their expertise. All identifiable information will be removed.

Experimental Human Pneumoccocal Challenge Model (EHPC)

Contact details

General questions: please contact the research team on

07740 410 290

during normal working hours.

Web site: https://www.lstmed.ac.uk/arc-volunteer-database

Emergency contact details at any time day or night: Mobile: 07912 053 981

The Chief Investigator for this study is **Dr Andrea Collins**. You may contact her at the Liverpool School of Tropical Medicine, Liverpool Life Sciences Accelerator Building, 1 Daulby Street, Liverpool, L7 8XZ, UK. Telephone: 0151 702 9439.

This research is sponsored by the Liverpool School of Tropical Medicine. It is funded by Merck. The research has been reviewed for scientific content by an external panel. The National Research Ethics Service Committee Liverpool Central has reviewed the study and given approval for it to take place.

Data protection: *If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.*

Data access: *The only people in LSTM who will have access to information that identifies you will be people who need to contact you to regarding your participation in the research or audit the data collection process. LSTM will use your name, NHS number and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from LSTM and regulatory organisations may look at your medical and research records to check the accuracy of the research study. LSTM (research site) will pass these details to LSTM (sponsor) along with the information collected from you and your medical records. The people who analyse the information will not be able to identify you and will not be able to find out your name, NHS number or contact details.*

LSTM (research site) will keep identifiable information about you from this study for a minimum of 5 years after the study has finished.

Information for research: *The information will only be used for the purpose of research and cannot be used to contact you or to affect your health care. It will not be used to make decisions about future services available to you, such as insurance.*

ARC ACCELERATOR RESEARCH CLINIC

LSTM LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Experimental Human Pneumoccocal Challenge Model (EHPC)

Consent Form

Participant Study number

Challenge 3 Study:

Please initial the box if you agree with each statement. Then, print and sign and date below.

I have read and understand the information sheet version ____**dated__/___/____** for the above study. I have been able to consider the information and ask questions. I confirm that the study procedures and information have been explained to me.	Initial
I understand that this study is voluntary and that I am free to withdraw without giving any reason without my medical care or legal rights being affected.	Initial
I understand that research notes relevant to taking part in this research and data collected may be seen by the regulatory authorities. I give permission for these people to access my records.	Initial
I agree that anonymous data and samples can be transferred outside of the UK for the purpose of the research.	Initial
I agree to my GP being informed of my participation and providing information or for the researcher to access my electronic GP summary record relevant to the study.	Initial
I understand that the samples collected will be anonymised and used and stored for the research described above, and that samples may be sent to national and international collaborating laboratories as part of the study.	Initial
I gift my samples to be used for future research in the UK and overseas. I understand that my samples are anonymised and will be transferred to a research tissue bank at the end of the study. I agree that my samples may be used in future research to investigate factors affecting infection and immunity. ……	Initial
I give permission for the study team to store my contact details in order to invite me to participate in future research	Initial
Biologically female participants of child-bearing potential: I confirm that I am not planning to conceive, and I will use effective contraception if required during the study.	Initial N/A ☐
I agree to take part in this study.	Initial

……	……	_ _/_ _ _/_ _ _ _
Name of participant (print)	Signature	Date
……	……	_ _/_ _ _/ _ _ _ _
Name of person receiving consent	Signature	Date

Experimental Human Pneumoccocal Challenge Model (EHPC)

Copies: 1 for participant, original for site file and one scanned or filed in research case notes

Supplemental Material 2. Biological Specimens

Biological samples are collected from all participants, transferred to the laboratory with an accompanying inventory form and stored, following local standard operating procedures.

The samples are “anonymised” with a participant number at the point of collection. Anonymised samples may be sent to national and international collaborators for further analysis, as detailed in the participant consent form. LSTM may store samples for up to 25yrs. After this time, the remaining samples will be transferred to a research tissue bank (LSTM).

Reporting checklist for protocol of a clinical trial.

Based on the SPIRIT guidelines.

Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the SPIRITreporting guidelines, and cite them as:

Chan A-W, Tetzlaff JM, Gøtzsche PC, Altman DG, Mann H, Berlin J, Dickersin K, Hróbjartsson A, Schulz KF, Parulekar WR, Krleža-Jerić K, Laupacis A, Moher D. SPIRIT 2013 Explanation and Elaboration: Guidance for protocols of clinical trials. BMJ. 2013;346:e7586

		Reporting Item	Page Number
Administrative information			
Title	#1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	#2a	Trial identifier and registry name. If not yet registered,	2

		name of intended registry	
Trial registration: data set	#2b	All items from the World Health Organization Trial Registration Data Set	1, 2, 4, 6, 7, 8, 9, 12, 15, 16
Protocol version	#3	Date and version identifier	2
Funding	#4	Sources and types of financial, material, and other support	18
Roles and responsibilities: contributorship	#5a	Names, affiliations, and roles of protocol contributors	Supp for editors
Roles and responsibilities: sponsor contact information	#5b	Name and contact information for the trial sponsor	1
Roles and responsibilities: sponsor and funder	#5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Supp for editors
Roles and responsibilities: committees	#5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and	Supp for editors

		other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	
Introduction			
Background and rationale	#6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4-6
Background and rationale: choice of comparators	#6b	Explanation for choice of comparators	N/A – no comparator used
Objectives	#7	Specific objectives or hypotheses	6-8
Trial design	#8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, non-inferiority, exploratory)	5
Methods: Participants, interventions, and outcomes			
Study setting	#9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can	6

		be obtained	
Eligibility criteria	#10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	8-10
Interventions: description	#11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10-11
Interventions: modifications	#11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving / worsening disease)	10
Interventions: adherance	#11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return; laboratory tests)	12
Interventions: concomitant care	#11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	9
Outcomes	#12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	6-8

Participant timeline	#13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	10-11, Figures1-3
Sample size	#14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	12-13
Recruitment	#15	Strategies for achieving adequate participant enrolment to reach target sample size	10
Methods: Assignment of interventions (for controlled trials)			
Allocation: sequence generation	#16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	N/A – no blinding used
Allocation concealment mechanism	#16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the	N/A – no blinding used

		sequence until interventions are assigned	
Allocation: implementation	#16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	N/A – no blinding used
Blinding (masking)	#17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A – no blinding used
Blinding (masking): emergency unblinding	#17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A – no blinding used
Methods: Data collection, management, and analysis			
Data collection plan	#18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	12
Data collection plan: retention	#18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be	12

		collected for participants who discontinue or deviate from intervention protocols	
Data management	#19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	16
Statistics: outcomes	#20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	13-14
Statistics: additional analyses	#20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	13-14
Statistics: analysis population and missing data	#20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	13-14
Methods: Monitoring			
Data monitoring: formal committee	#21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is	Supp for editors

		not needed	
Data monitoring: interim analysis	#21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	14
Harms	#22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	14-15
Auditing	#23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	15
Ethics and dissemination			
Research ethics approval	#24	Plans for seeking research ethics committee / institutional review board (REC / IRB) approval	15
Protocol amendments	#25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC / IRBs, trial participants, trial registries, journals, regulators)	15
Consent or assent	#26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and	15-16

		how (see Item 32)	
Consent or assent: ancillary studies	#26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	16
Confidentiality	#27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	16
Declaration of interests	#28	Financial and other competing interests for principal investigators for the overall trial and each study site	17
Data access	#29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	16
Ancillary and post trial care	#30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	16
Dissemination policy: trial results	#31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	16
Dissemination policy:	#31b	Authorship eligibility guidelines and any intended use of	16

authorship		professional writers	
Dissemination policy: reproducible research	#31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	16

Appendices

Informed consent materials	#32	Model consent form and other related documentation given to participants and authorised surrogates	Supp 1
Biological specimens	#33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	Supp 2