Statistical Analysis Plan

MARVELS 1.0

Feasibility study of Experimental Human Pneumococcal Carriage in Malawi

Pneumococcal Controlled Human Infection Model in Malawi: Transfer of an Established Pneumococcal Carriage Model from Liverpool, UK to Blantyre, Malawi – A Feasibility Study

SAP version: v1.3

SAP date: 12 January 2021

SAP Author: Dr. Marc Henrion (with input from the study team)

Corresponding study protocol version: v1.6 (10 September 2019)

Table of Contents

SIGNATURE PAGE

Statistical Analysis Plan (v1.0)

Pneumococcal Controlled Human Infection Model in Malawi: Transfer of an Established Pneumococcal Carriage Model from Liverpool, UK to Blantyre, Malawi – A Feasibility Study (MARVELS 1.0)

SPONSOR

Liverpool School of Tropical Medicine (LSTM)

PROTOCOL NUMBER

MARVELS 1.0 (v1.6)

Principal Investigator: Prof. Stephen Gordon

Signed: _____ Date: _____

Study Statistician: Dr. Marc Henrion

Signed: _____ Date: _____

1 Preface

This Statistical Analysis Plan (SAP) for "Pneumococcal Controlled Human Infection Model in Malawi: Transfer of an Established Pneumococcal Carriage Model from Liverpool, UK to Blantyre, Malawi – A Feasibility Study" (MARVELS 1.0) describes and expands upon the statistical information presented in the study protocol.

This document describes all planned analyses and provides reasons and justifications for these analyses. This SAP will follow internationally accepted guidelines published by the American Statistical Association[1] and the Royal Statistical Society[2] for statistical practice.

This document contains a review of the study design, general statistical considerations, comprehensive statistical analysis methods. Any deviation from this SAP will be described and justified in the final study report. The reader of this SAP is encouraged to also review the study protocol for details on conduct of the study.

2 Investigators

Prof. Stephen Gordon (Principal Investigator and TSC)
Director, Malawi-Liverpool-Wellcome Trust Clinical Research Programme
Professor, Liverpool School of Tropical Medicine

Dr. Kondwani Jambo (Investigator, TSC)
Head, Viral Immunology, Malawi-Liverpool-Wellcome Trust Clinical Research Programme
Lecturer, Liverpool School of Tropical Medicine

Dr. Marc Henrion (Investigator, TSC)
Head, Statistical Support Unit, Malawi-Liverpool-Wellcome Clinical Research Programme
Senior biostatistician, Liverpool School of Tropical Medicine

Dr. Ndaziona Peter Banda (Investigator)
Consultant, Respiratory Medicine, Queen Elizabeth Central Hospital

Dr Ben Morton (Investigator)
Senior clinical lecturer, Malawi-Liverpool-Wellcome Clinical Research Programme & Liverpool School of Tropical Medicine

Dr. Jamie Rylance (Investigator)
Head, Lung Health, Malawi - Liverpool School of Tropical Medicine
Reader, Liverpool School of Tropical Medicine

Blessings Kapumba (Investigator)
Malawi-Liverpool-Wellcome Clinical Research Programme

Dr. Sarah Burr (Investigator)
Post-doctoral scientist, Malawi-Liverpool-Wellcome Clinical Research Programme & Liverpool School of Tropical Medicine

Edna Nsomba (Investigator)
Senior research nurse, Malawi-Liverpool-Wellcome Trust Clinical Research Programme & Queen Elizabeth Central Hospital

Clemens Masesa (Investigator)
Head, Data Research Support Unit, Malawi-Liverpool-Wellcome Trust Clinical Research Programme
Senior data systems manager, Liverpool School of Tropical Medicine

Prof. Daniela Ferreira (Collaborator)
Dean, Department of Clinical Sciences, Liverpool School of Tropical Medicine

3 List of abbreviations

AE	Adverse Event
ATP	According to Protocol
BMI	Body Mass Index
CAP	Community Acquired Pneumonia
CFU	Colony Forming Units
CHIM	Controlled Human Infection Model
CRF	Case Report Form
DSMB	Data, Safety and Monitoring Board
EHPC	Experimental Human Pneumococcal Colonisation
ELISA	Enzyme-Linked Immunosorbent Assay
ELISPOT	Enzyme-Linked Immune Absorbent Spot
FBC	Full Blood Count
GCP	Good Clinical Practice
HIV	Human Immunodeficiency Virus
HTS	Human immunodeficiency virus Testing Service
ICH-GCP	International Conference on Harmonisation of Good Clinical Practice
ITT	Intention to Treat
LSTM	Liverpool School of Tropical Medicine
MARVELS	Malawi Accelerated Research in Vaccines, Experimental and Laboratory Systems
MHRA	Medicines and Healthcare products Regulatory Authority (UK Regulator)
MK	Malawian Kwacha
MLW	Malawi-Liverpool-Wellcome Trust Clinical Research Programme
MTA	Material Transfer Agreement
NHSRC	National Health Sciences Research Committee
OM	Otitis Media
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase Chain Reaction
PCV	Pneumococcal conjugate vaccines
PCV13	Pneumococcal conjugate vaccine 13

PCVPA	Pneumococcal Carriage in Vulnerable Populations in Africa
PMPB	Pharmacy, Medicines and Poisons Board
QECH	Queen Elizabeth Central Hospital
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SOP	Standardised Operating Procedure
SUSAR	Serious Unexpected Serious Adverse Reaction
TSC	Trial Steering Committee

4 Introduction

This document describes the study design, sample size and statistical analyses for the MARVELS 1.0 study.

This study aims to accelerate pneumococcal vaccine research by transferring a safe and established controlled human infection model (CHIM) from the Experimental Human Pneumococcal Colonisation (EHPC) programme in Liverpool to a Malawian setting. The Liverpool CHIM has been used in over 1000 healthy adults for the past nine years safely with no cases of active infection or related serious adverse effects. A Malawian pneumococcal CHIM will allow targeted pneumococcal vaccine candidate choices to be made based on relevant pathogen challenge in an at-risk population who stand the most to gain from new and improved vaccine strategies.

The Liverpool CHIM was established to test the effect of new candidate vaccines with significant cost and time savings compared with phase III clinical trials[3]. However, a valid criticism of current vaccine research strategies is that these have been conducted in geographical areas and participant populations not at significant risk of disease. MARVELS 1.0 is the first step in addressing this, by assessing the safety and feasibility of transferring the Liverpool CHIM to Malawi.

5 Study aims, outcomes

5.1 Study Aims

The aims of this study are:

PRIMARY

1. Establish controlled human infection with *Streptococcus pneumoniae* serotype 6B in Malawi.
2. Confirm nasopharyngeal pneumococcal challenge dose required to establish ~50% carriage in Malawian participants.
3. Confirm safety and measure potential symptoms of controlled human infection procedures for study participants.

SECONDARY

4. Confirm sampling protocols and laboratory assays for immunological assessment relevant to vaccine testing.

TERTIARY

5. Assess the feasibility and acceptability of consent and study procedures for participants.

This SAP concerns aims 2 and 3. Aims 1 and 4 do not require statistical analysis and aim 5 is assessed by qualitative methods.

5.2 Study Hypothesis

That inoculation of Malawian volunteers with 80,000 cfu/naris will safely result in nasopharyngeal carriage in ~50% of participants as it has done in Liverpool.

Study Design

A feasibility study of adult healthy human participants experimentally exposed to escalating doses of *Streptococcus pneumoniae* in the nasopharynx to determine optimal dosage for establishment of nasal carriage. We will closely monitor study participants to ensure safety and tolerability of study procedures. We will measure immune protective responses to *Streptococcus pneumoniae* challenge using mucosal (nasal, throat and salivary) and blood samples to determine assay feasibility.

The study will be carried out in four stages:

1. **Screening and recruitment:** Potential participants will be screened to ensure health and safety as detailed in Methods.
2. **Inoculation with pneumococci:** Participants will be randomly allocated to inoculation with *Streptococcus pneumoniae* serotype 6B or 0.9% saline (sham inoculation) to the inside of each naris. Participants will be monitored for safety and establishment of carriage. The dose escalation schedule is described in Figure 6.1.

3. **Detection of pneumococcal carriage:** Nasal wash samples will be taken, according to a standardized protocol, at days 2, 7, and 14 days post inoculation. Classical microbiological culture will determine nasal colonisation with pneumococcal serotype 6B at each time point.
4. **Immunology response measurements:** exploratory blood, mucosal and nasal cell samples will be taken to confirm robust clinical and laboratory standard operating procedures and explore the immunological response to nasal challenge.

5.3 Study endpoints

5.3.1 Feasibility endpoints

Primary endpoint: Binary recording of nasal colonisation by *Streptococcus pneumoniae* serotype 6B by day 14 following pneumococcal challenge. This is defined as the detection of the inoculated pneumococci by classical culture methods, at any time point, from nasal wash recovered from the participants at days 2, 7 and 14 following pneumococcal challenge.

Secondary endpoints: Confirmation of robust clinical and laboratory methods for sample capture and processing. Detection of *Streptococcus pneumoniae* by lytA qPCR at any time point from nasal wash recovered from the participants at days 2, 7 and 14 following pneumococcal challenge.

Tertiary endpoints: Acceptability of study and methods.

5.3.2 Safety endpoints

Adverse events (AEs), serious adverse events (SAEs) and suspected unexpected serious adverse reactions (SUSARs) as defined by ICH-GCP will be recorded systematically.

5.4 Study Setting

Clinical procedures will be conducted at the Queen Elizabeth Central Hospital Research Ward, Blantyre, Malawi. Laboratory procedures will be conducted at the adjacent Malawi-Liverpool-Wellcome Trust Clinical Research Programme (MLW) Laboratories.

5.5 Recruitment Target

A maximum of 36 healthy adult participants will be recruited to complete the study.

5.6 Duration

Recruitment of all participants and follow up will be completed within 12 months. A Gantt chart is shown (in the following page), and the details of each section follow.

5.7 Participants, schedule and timelines

We will inoculate healthy non-smoking adult participants with a well-characterised, fully sequenced penicillin-sensitive pneumococci and observe them for the development of pneumococcal carriage up to 14 days post inoculation. Study discharge will occur on day 20.

Study visits must take place according to the proposed schedule to ensure participant safety. This will be clearly explained to the participants during recruitment and consent. If a

participant is unable to comply, they will not be recruited (Participant Flow Chart - Figure 5.1). Daily phone contact occurs between study visits until day 14.

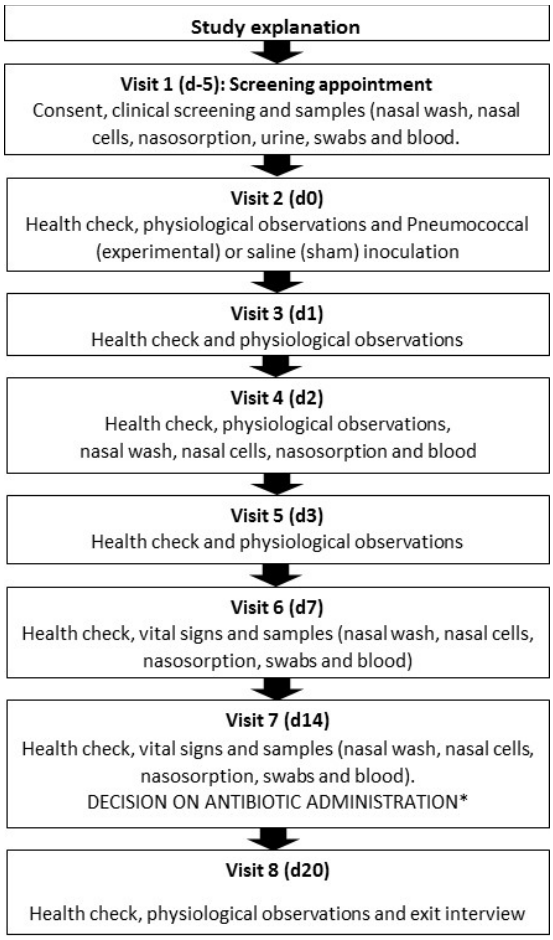

Figure 5.1 Participant flow chart. D-5 refers to 5 days before day 0, the day of inoculation, and d1 is one day after and so on. *The decision for antibiotics will be communicated directly at visit 7 except in the unlikely circumstance that only d14 is positive for carriage in which case the result will be telephoned in time for visit 8.

6 Investigational plan

6.1 Study design

The basic study design is that of a dose escalation study and summarised on Figure 6.1.

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 carriage at a dose in which it is unlikely to occur; b) maximise safety by inoculating small groups (in case adverse events occur) before continuing on to larger groups in which we will be able to give reasonable precision to estimate carriage rates. Groups 1, 2 and 3 will receive increasing doses of pneumococcal inoculation as explained in Figure 6.1. Briefly, 20,000 cfu/naris (Group 1) is not expected to achieve carriage. If 80,000 cfu/naris achieves more than 4 from 9 participants with carriage, then the study will end at that point. Sham inoculation with 0.9% normal saline has been incorporated into the study design to assess whether any potential observed effects in participants are pneumococcal challenge-related or simply reflect the study conditions.

Randomization has been included to prepare (test feasibility) for future vaccine studies. The clinical team and participants will be blinded to the allocation to avoid bias in the collection and evaluation of data during the study. The randomisation code will be produced by MLW Clinical Research Support Unit (Co-investigator Henrion) using a computer-generated pseudo-random permutation procedure. Prior to the start of the study, a copy of the master randomisation code will be supplied to the MLW laboratory team to enable the production of *Streptococcus pneumoniae* inoculations and sham inoculations as required by the randomisation schedule. The laboratory team will not be blinded to the challenge allocation, this measure is to ensure participant safety and monitoring during follow up. Research team members will be assigned solely to one of two separate delegation logs for blinded and unblinded activities (clinical and laboratory).

6.2 Randomisation

Randomization has been included to prepare (test feasibility) for future vaccine studies. The clinical team and participants will be blinded to the allocation to avoid bias in the collection and evaluation of data during the study. The randomisation code will be produced by MLW Clinical Research Support Unit (Co-investigator Henrion) using a computer-generated pseudo-random permutation procedure. Prior to the start of the study, a copy of the master randomisation code will be supplied to the MLW laboratory team to enable the production of *Streptococcus pneumoniae* inoculations and sham inoculations as required by the randomisation schedule. The laboratory team will not be blinded to the challenge allocation, this measure is to ensure participant safety and monitoring during follow up. Research team members will be assigned solely to one of two separate delegation logs for blinded and unblinded activities (clinical and laboratory).

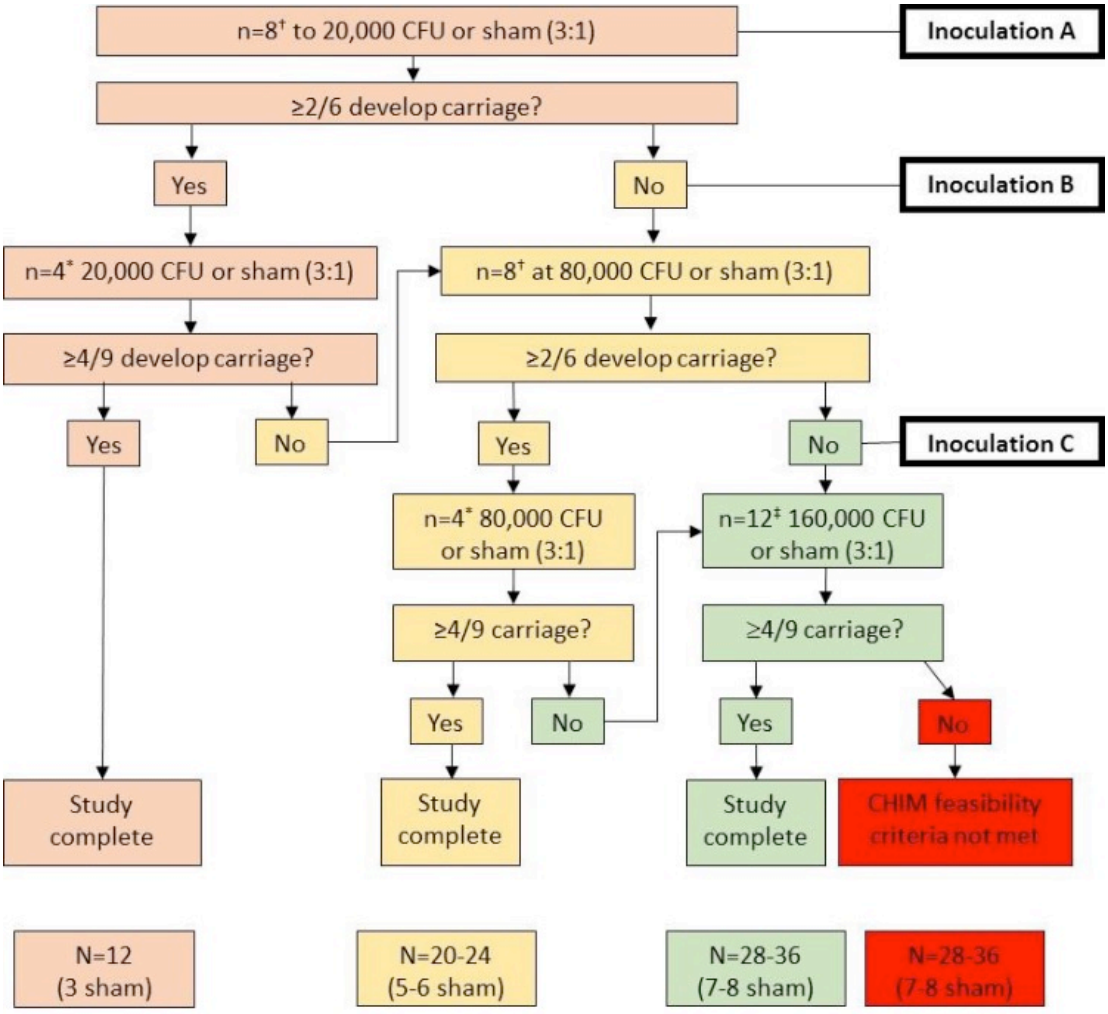

Figure 6.1: Dose escalation study design, adapted from Waddington et al[4]. The doses for Groups 1, 2 and 3 will be 20000, 80000 and 160000 pneumococcal colony forming units (CFU) per naris respectively. All participants will be monitored for adverse effects and for pneumococcal carriage. The first cohort will be inoculated with 20,000 CFU/naris (**Inoculation A**). **†**: Eight participants will be randomised 3:1 to pneumococcal inoculation (n=6) or sham inoculation with 0.9% saline (n=2). If ⍰2/6 participants from the initial cohort of eight develop experimental pneumococcal carriage, a further 4 participants will be recruited to this dose cohort. *****: Four participants will be randomised 3:1 to pneumococcal inoculation (n=3) or sham inoculation with 0.9% saline (n=1). If the combined carriage rate of the completed cohorts is ≥4/9 (6+3 nasally challenged with pneumococcus), then the nasal challenge dose will be established, and we will conclude the study. If at either of these decision points, the carriage rate is insufficient, the algorithm will be restarted with a higher challenge dose of 80,000 CFU/naris (**Inoculation B**) and the recruitment schedule repeated. If at 80,000 CFU, the carriage rate is insufficient, then an additional cohort of participants will be recruited to the 160,000CFU/naris dose escalation group (**Inoculation C**). **‡**: Twelve participants will be randomised 3:1 to pneumococcal inoculation (n=9) or sham inoculation with 0.9% saline (n=3). We have previously demonstrated 50-60% carriage rates with the 80,000 CFU 6B dose in the Liverpool pneumococcal controlled human infection model[4,5].

6.3 Determination of colonisation

Colonisation will be defined by the microbiology result of nasal washes taken at 2-, 7- and 14-days post inoculation. Nasal washes will be plated on to culture media and incubated overnight at 37°C in 5% carbon dioxide (CO2). Colonies will be confirmed as *S. pneumoniae* using classical microbiological techniques including (i) typical draughtsman-like colony morphology, (ii) the presence of α-haemolysis, (iii) optochin sensitivity and (iv) Gram-positive diplococci. Typing by latex agglutination will be done using a commercial kit to confirm pneumococcal serogroup. Isolates will be frozen at -80oC for storage. Results from the cultured nasal wash will also be confirmed using Polymerase Chain Reaction (PCR) based methods of bacterial detection.

Monitoring of colonisation will be performed by microbiology analysis of nasal washes.

6.4 Study Participants

Advertisements inviting healthy participants to participate will be widely placed. Areas will include physical notice boards, table display, electronic notice boards, the intranet/internet of local universities and colleges (with permission), social media, the local press, television and radio. Staff from the MLW team will be permitted to participate providing they are not members of the study team.

Interested persons will be asked to contact the research team by phone, email or text for further information. Once potential participants who are interested have contacted the research team, they will then be invited to meet a member of the research team. The research team will explain the study at the meeting and the Participant Information Sheet will be provided. Participants will then consider if they wish to continue (see Figure 5.1).

At visit 1, the informed consent process will be conducted by either the research nurse or research doctors who are formally delegated by the Chief Investigator, trained in GCP, consent and the trial protocol. Following consent, inclusion and exclusion criteria will be applied as below.

6.4.1 Inclusion criteria:

- Adults aged 18-40 years - ages chosen to minimise the risk of pneumococcal infection
- Fluent spoken and written Chichewa or English - to ensure a comprehensive understanding of the research project, their proposed involvement and communication with all members of the research team

6.4.2 Exclusion criteria:

- Previous pneumococcal vaccination
- HIV-infection: HIV testing will be performed by trained personnel certified in HIV Testing Services (HTS). “Determine” or “Unigold” will be used as the first line tests. Any participants identified as HIV-infection positive will be referred to the governmental system for infection confirmation, treatment and follow up.

- Close physical contact (e.g. sleeping in the same room or nursing) with at risk individuals (children under 5 years age, immunosuppressed adults, elderly, chronic ill health)
- Allergy to penicillin/amoxicillin
- Acute illness
    - Current illness
    - Acute illness within 3 days prior to inoculation
    - Antibiotic treatment within 2 weeks of inoculation
- Chronic illness that may impair immune response or impair ability to comply with study procedures and safety monitoring (e.g. HIV, diabetes).
- Taking immunosuppressive medication that may include but are not limited to steroids and steroid nasal spray.
- Pregnancy - minimise risk of pneumococcal disease
- Involved in another clinical trial unless observational or in follow-up (non-interventional) phase
- History of drug or alcohol abuse. This is very difficult to ascertain in a history therefore we will exclude people reporting either i) drinking alcohol more than twice per week, ii) more than 14 units per week (if female), more than 21 units per week (if male).
- History of Smoking
    - Current regular smoker (smokes daily/ smokes > 5 cigarettes per week) - minimise risk of pneumococcal disease
    - Recent smoker i.e. within the last 6 months - minimise risk of pneumococcal disease
    - Ex-smoker with a significant smoking history (>10 pack years) – minimise risk of pneumococcal disease
- Unable to give informed consent
- In case of any uncertainty or concern, the PI will take clinical responsibility for the decision.

6.5 Analysis Populations

Intention-to-Treat Analysis (ITT) Population: The ITT population will consist of all randomized subjects. For analysis purposes, ITT subjects are analysed as part of the study arm (sham, *Streptococcus pneumoniae* 20,000 CFU*, Streptococcus pneumoniae* 80,000 CFU*, Streptococcus pneumoniae* 160,000 CFU) they have been randomized to.

Safety Population: The safety population will consist of all subjects who received a *Streptococcus pneumoniae* inoculation (at any of the 3 study doses).

According to Protocol (ATP) Population: The ATP population will consist of all ITT population subjects that have received an inoculation (sham or *Streptococcus pneumoniae*) and without

major protocol violations. For analysis purposes, ATP subjects are analysed as part of the study arm according to which inoculation and dose they actually received. Participants that are withdrawn from the study and replaced by another participant will not be part of the ATP population.

As this is a feasibility study, the ATP population will be the main analysis population for assessing feasibility of the Malawi CHIM. For safety endpoints, the safety population will be the main analysis population.

6.6 Protocol Deviations

A summary of subject-specific protocol deviations will be presented by the reason for the deviation, the deviation category, and treatment group for all subjects (Listing 6.1). Deviations that are considered major deviations will be reviewed by the PI prior to interim and final analysis for possible subject exclusion from the ATP population. Major deviations include the informed consent not being signed prior to study procedures, unblinding of treatment, and becoming aware that an enrolled subject failed to meet inclusion criterion or met exclusion criterion. Non-subject specific protocol deviations will also be summarized (Listing 6.2).

6.7 Data Collection

Following consent, inclusion and exclusion criteria, data and samples will be collected.

- **Clinical examination** - the initial clinic visit will include a focused clinical history and targeted clinical examination involving auscultation of the lung fields and heart sounds. Participants will be informed if their clinical examination is normal. Should a previously unrecognised abnormality be identified, this will be explained to the individual, and appropriate investigations and follow-up will then be arranged by the study team. Further participation will be determined at the discretion of the study doctor dependent on the nature of the abnormality detected. If the participant is not eligible due to an acute illness, they may be re-screened at a later date with their verbal consent.
- **Nasal wash** - will be performed using a modified Naclerio method[6]. This is a well-used and validated technique to collect nasal bacterial specimens used successfully by the Liverpool CHIM team who will rigorously train the Malawi team. Briefly, 5ml of sterile saline is instilled and held for a few seconds in the nares before being expelled in to a sterile Galli pot. This is repeated twice in each naris using 20ml saline in total. In the event of nasal wash loss (defined as cough/sneeze/swallow), the procedure may then be repeated to obtain an adequate specimen. Should the nasal wash reveal natural colonisation of pneumococcus, the participant will continue in the study and receive the inoculation as per protocol, unless the natural serotype is identified as 6B, in which case the patient then leaves the study at day 2. All pneumococcal carriage negative participants will continue in the study. After the inoculation visit, nasal washes will then be taken at 2-, 7- and 14-days post inoculation.
- **Nasosorption strips** - Nasosorption will be obtained before a nasal wash and/or inoculation. The strips are like blotting paper developed by Hunts Development Ltd (UK). They collect concentrated nasal lining fluid before the nasal wash to measure

inflammatory responses induced by infection that may be associated with increased colonisation density and acquisition. Concentrate nasal fluid will be used to measure cytokine levels by multiplex bead array. Blotting paper will be held inside the nostril for up to 3 minutes until soaked, removed and placed in the microcentrifuge tube for storage.

- **Nasal Cells** –will be collected using a nanosampling method in which cells are obtained through minimally invasive superficial nasal scrape biopsies (rhinoprobe). Work from our Liverpool team demonstrates that participants can be biopsied multiple times with no significant side effects. Nasal cell samples will be obtained after nasal wash samples have been taken. Up to 4 samples (2 per nostril) will be obtained at each nasal sampling visit. If the researcher finds that the sample is insufficient, for example no cells are visible on the rhinoprobe, the sample can be repeated immediately. The rhinoprobes are placed in transport media immediately after collection.
- **Blood** – blood samples will be obtained using venepuncture by an appropriately trained team member. In the entire duration of the study, a maximum of 100mL of blood will be collected across the entire study for analysis including a full blood count. The volume of blood collected for each laboratory test is detailed in Table 2 below. Participants with an abnormal full blood count will be removed from the study as determined by the clinical team (result day 2).
- **Throat swabs** – simple posterior pharyngeal swabbing on a dry swab.
- **Saliva** – salivette held for saturation or a spit in the tube.
- **Urine Sample -** for a pregnancy test as part of the safety screening.

6.8 Sample Size and Statistical Power

This is a feasibility study that transfers a proven existing technique therefore we have not formally calculated power to detect the carriage rate. The study protocol and sample size are based on our previously published study demonstrating 50% nasal carriage at the 80,000 CFU dose in Liverpool, UK[5]. This feasibility study is designed to determine if experimental pneumococcal nasal carriage is possible in the Malawian setting.

Depending on the carriage rate, up to 36 participants (Figure 6.1) will be recruited, with 3:1 random allocation to *Streptococcus pneumoniae* and sham inoculation.

7 General considerations

The reporting of this study will be prepared in accordance to the CONSORT 2010[7] guidelines

All continuous data variables will be summarized using the following descriptive statistics: N (size of relevant analysis population), n (size of analysis population without missing values), arithmetic / geometric mean, standard deviation (SD), median, 25th percentile value (P25), 75th percentile value (P75) and interquartile range (IQR), minimum and maximum. The geometric mean will be reported for log-transformed variables. The proportion of observed levels will be reported for all binary and categorical measures. When appropriate, corresponding exact 95% confidence intervals (CIs) for proportions will be included.

7.1 Timing of analyses

The primary feasibility endpoint of nasal colonisation will be assessed at least 2 times and up to 5 times given the adopted dose escalation design (Figure 6.1):

1. After the last of the first 8 participants has concluded visit 7 (d14).
2. If more than 2 of the 6 *Streptococcus pneumoniae* inoculated participants (dose 20,000 CFU) are colonised: after the last of the next 4 participants at this dose has completed visit 7 (d14).
3. If the study proceeds to the 80,000 CFU inoculation dose: after the last of the 8 participants has concluded visit 7 (d14).
4. If more than 2 of the 6 *Streptococcus pneumoniae* inoculated participants (dose 80,000 CFU) are colonised: after the last of the next 4 participants at this dose has completed visit 7 (d14).
5. If the study proceeds to the 160,000 CFU inoculation dose: after the last of the 12 participants has concluded visit 7 (d14).

All other feasibility analyses will be conducted at the completion of the study.

Safety endpoints will be monitored continuously throughout the study and will be summarised at the completion of the study.

7.2 Missing data

Due to the close monitoring of study participants, with secondary contact details available for every participant, few missing data are anticipated. Still, study participants may withdraw from the study, and missing data may originate due to a variety of reasons.

For the primary feasibility endpoint, data will be available for as many participants as given by the study design from Figure 6.1 since an alternative participant will be recruited in the case of withdrawal of a participant. Participants missing study visits so that no day 2, 7 or 14 data is available will be withdrawn from the study and their data excluded from data analysis.

A new participant will be recruited in such a case and this new participant will be allocated to the same pneumococcus / sham inoculation as the participant ID for the withdrawn participant was randomised to. The primary feasibility endpoint will be assessed, at each of the five analysis time points using colonisation results data from all participants that are required for that stage's analysis and that have completed visit 7 (d14).

Other analyses are primarily descriptive or make use of censored data techniques, so that no advanced missing data methods will be used in these analyses.

7.3 Statistical significance and multiple testing

Success (feasibility) of the study will be decided according to the decision rules stated on Figure 6.1.

Success (safety) of the study will de decided by decision of the DSMB & TC upon revision of each SAE and SUSAR.

For this reason, no formal null hypothesis significance testing will be performed for the main analysis and no multiple testing adjustment will need to be made.

For secondary analyses, statistical significance is defined as $p<0.05$, but effect sizes / parameter estimates, exact p-values and confidence intervals will be stated. While we do report on statistical significance, results will be presented and discussed in accordance with recent guidance[8].

7.4 Reporting conventions

P-values ≥0.001 and ≤0.999 will be reported to 3 decimal places; p-values less than 0.001 will be reported as "<0.001". The mean, standard deviation, median, IQR and other statistics will be reported to one decimal place greater than the original data. Minimum and maximum values will use the same number of decimal places as the original data. Proportions will be presented as two decimal places; values greater than zero but <0.01 will be presented as "<0.01". Percentages will be reported to 2 decimal places; values greater than zero but < 1% will be presented as "<1%"; values greater than 99% but less than 100% will be reported as >99%. Estimated parameters, not on the same scale as raw observations (e.g. regression coefficients) will be reported to 3 significant figures. Inoculation dose concentrations and carriage densities will be reported in CFU/µl.

7.5 Technical details

All analyses and figures will be performed using the R environment for statistical computing and graphics[9] and associated packages.

8 Analysis

Results will be published in accordance with the CONSORT 2010 statement. A CONSORT diagram will summarise participant screening, enrolment, randomisation, inoculation, withdrawals, follow-ups and analysis (Figure 8.1).

8.1 Feasibility

The primary analysis aims to establish feasibility of the Malawi CHIM: does one of the 3 doses (20,00, 80,000, 160,000 CFU) lead to ≥50% nasal colonisation?

To assess this, the decision algorithm specified in the study design (Figure 6.1) is followed and no statistical analysis is required.

However, we will complement this with a number of descriptive and secondary analyses:

For each inoculation dose used in the study, we will estimate colonisation rates (by serotype 6B and by serotypes other than 6B) and compute 95% exact, binomial confidence intervals (Table 8.1). We will also compute this for participants inoculated with the sham, saline solution.

If more than 1 dose is used in the study, we will plot the estimated colonisation rate against inoculation dose for a crude estimate of the dose-response curve (Figure 8.2).

For each inoculation dose, and for serotype 6B only, we will plot time curves of colonisation rates at visits 0 (d-5), 4 (d2), 6 (d7), 7 (d14) (Figure 8.3).

We will also use lytA qPCR to assess nasal carriage by *Streptococcus penumoniae*. We will repeat all analyses using this method of carriage detection. In addition, we will formally compare carriage rates as determined by culture and PCR at days 2, 7 and 14 after pneumococcal challenge. We will list the 2x2 confusion matrices for each time point (Table 8.2) and, using only records with both measurements, compute the concordance proportions and their associated exact 95% confidence intervals, both overall and per inoculation group (Table 8.3). Further, for the qPCR results, we will estimate the geometric mean carriage densities at each of visits 4, 6 and 7 and associated 95% confidence intervals for each challenge dose; only data from colonised participants will be used in these calculations (Table 8.4, Figure 8.4). If more than one dose is used, we will also plot individual carriage densities against inoculation dose for a crude estimate of the carriage density dose response curve (Figure 8.5). If needed, we will estimate the lower limits of detection and quantification for the qPCR data, then use censored data techniques to account for the uncertainty associated with values below these limits. We will list recorded carriage durations (Table 8.5). We will also calculate the mean actual inoculation dose (as measured just before and after each inoculation) and report this together with estimates of the standard deviations for each planned inoculation dose (Table 8.6). To interrogate whether the volume of nasal wash returned from each participant has an effect on the carriage density measurement, we will plot density of colonisation against the volume of nasal wash returned, stratified by inoculation dose (Figure 8.6).

We will also compile scatter plots of temperature (both minimum and maximum) and difference in pre- and post-inoculation densities of the prepared inocula (Figures 8.7, 8.8) and a smooth regression model (locally estimated scatterplot smoothing, LOESS) will be fitted to descriptively assess whether temperature has had an effect on the inocula concentrations. Similarly, we will produce a scatter plot and fit a linear regression model of time difference (in minutes) from inoculum preparation to inoculum plating post-inoculation and difference in pre- and post-inoculation densities of the prepared inocula to assess whether time impacts CFU (Figure 8.9).

We will compile a summary table for demographic (age, sex, smoking status, alcohol consumption), baseline physiological (pulse rate, oxygen saturation on room air, systolic blood pressure, diastolic blood pressure, tympanic body temperature, height, weight, BMI, haemoglobin, white blood cell count, baseline carriage of *S. pneumoniae* other than serotype 6B) and baseline immunological variables (Tables 8.7, 8.8). Baseline physiological and immunological variables are those measured at the inoculation visit or for which the sample was taken at the inoculation visit (prior to the inoculation).

Analogous tables will be compiled for the physiological assessments made at visits 2-8 (Tables 8.9-8.15) and immunological measurements made at visits 4, 6 and 7 (Tables 8.16-8.18).

8.2 Safety

AEs, SAEs, SUSARs are monitored throughout the study and any decision to stop the trial is taken by the TSC in consultation with the DSMB.

At study end, AEs, SAEs and SUSARs will be listed by type and by inoculation group (sham, *Streptococcus pneumoniae* 20,000 CFU, 80,000 CFU, 160,000 CFU) and by carriage status (Listing 8.1). We will also stratify these lists by sex (Listing 8.1). We will also compile a full listing of all AEs, SAEs, SARs, SUSARs experienced by each participant (Listing 8.2).

Symptoms reported by study participants will also be listed in Listing 8.1. The following symptoms will be reported:

- Sore throat
- Coryzal symptoms
- Earache
- Feverish
- Cough
- Headache
- New Rash
- Other

Participants withdrawn from the study and excluded from analysis will also be listed (Listing 8.3).

8.3 Mock Figures

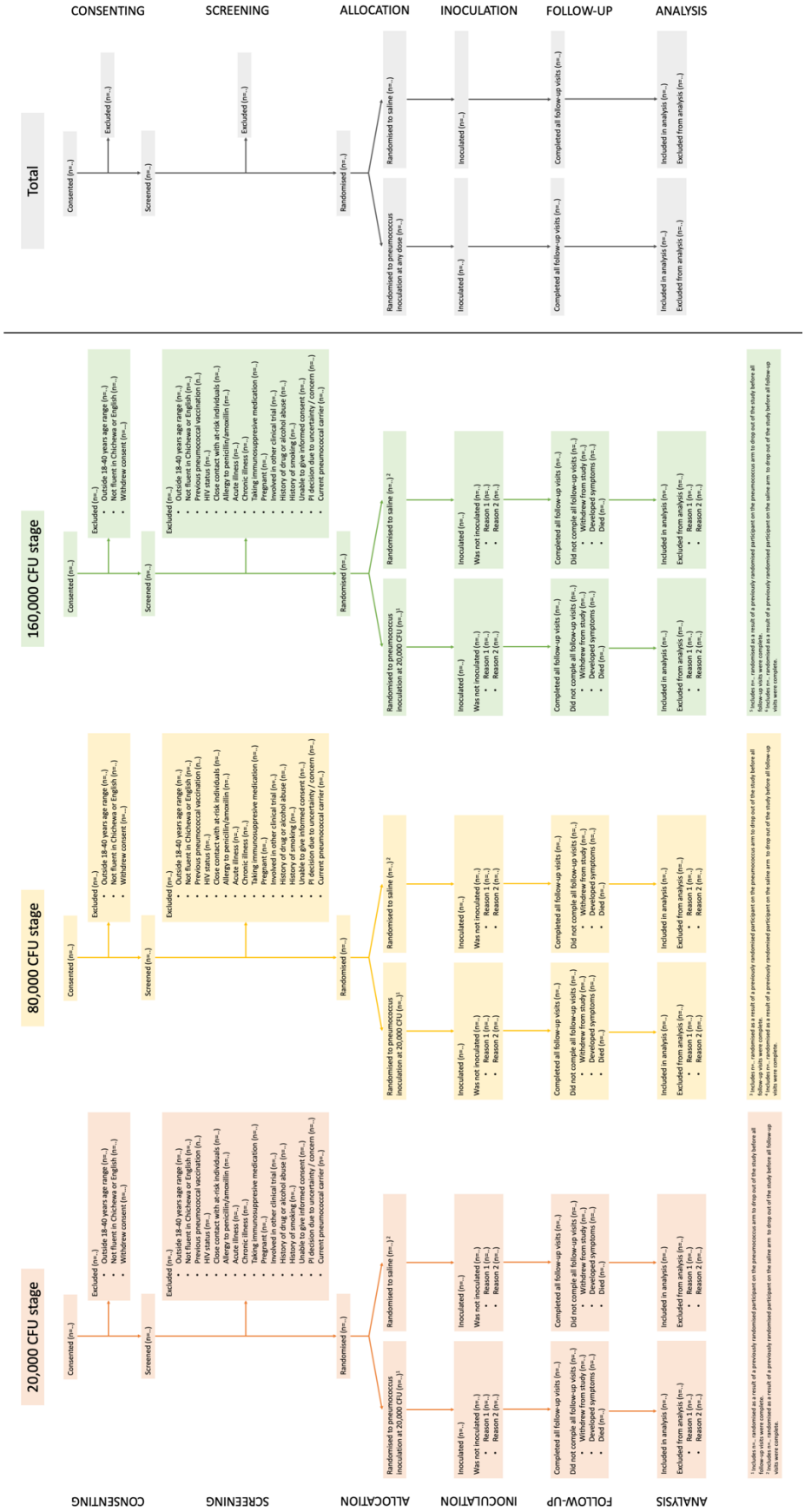

Figure 8.1: CONSORT diagram

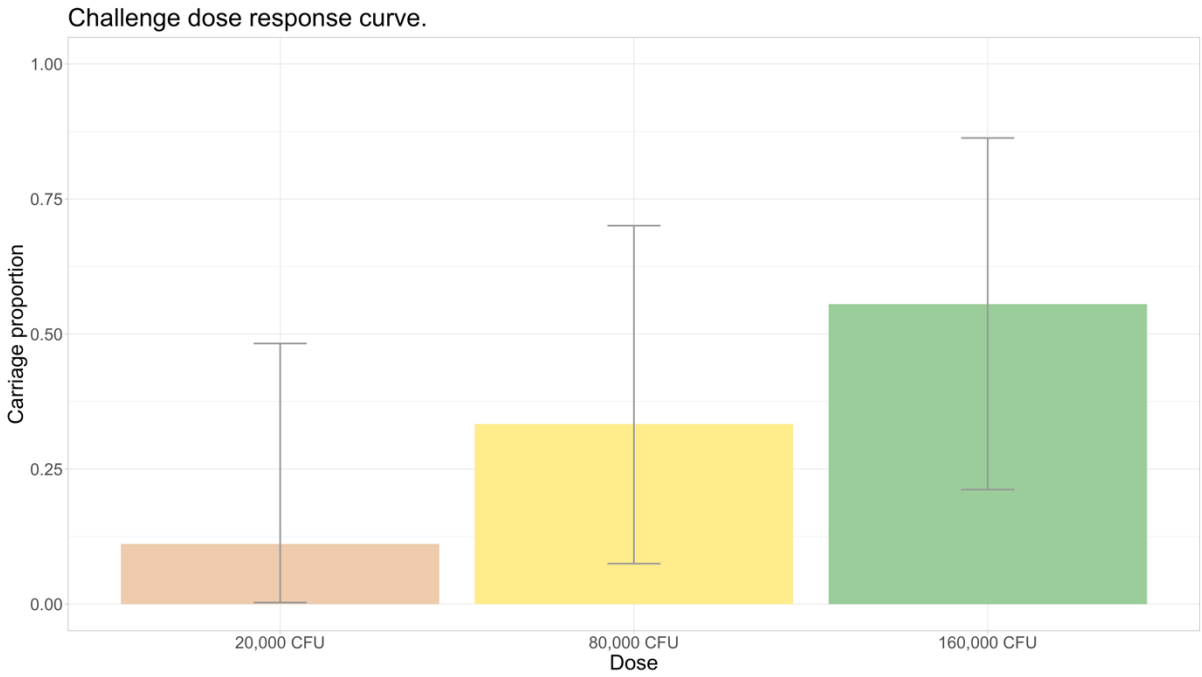

Figure 8.2: Challenge dose response curve (in terms of carriage proportion).

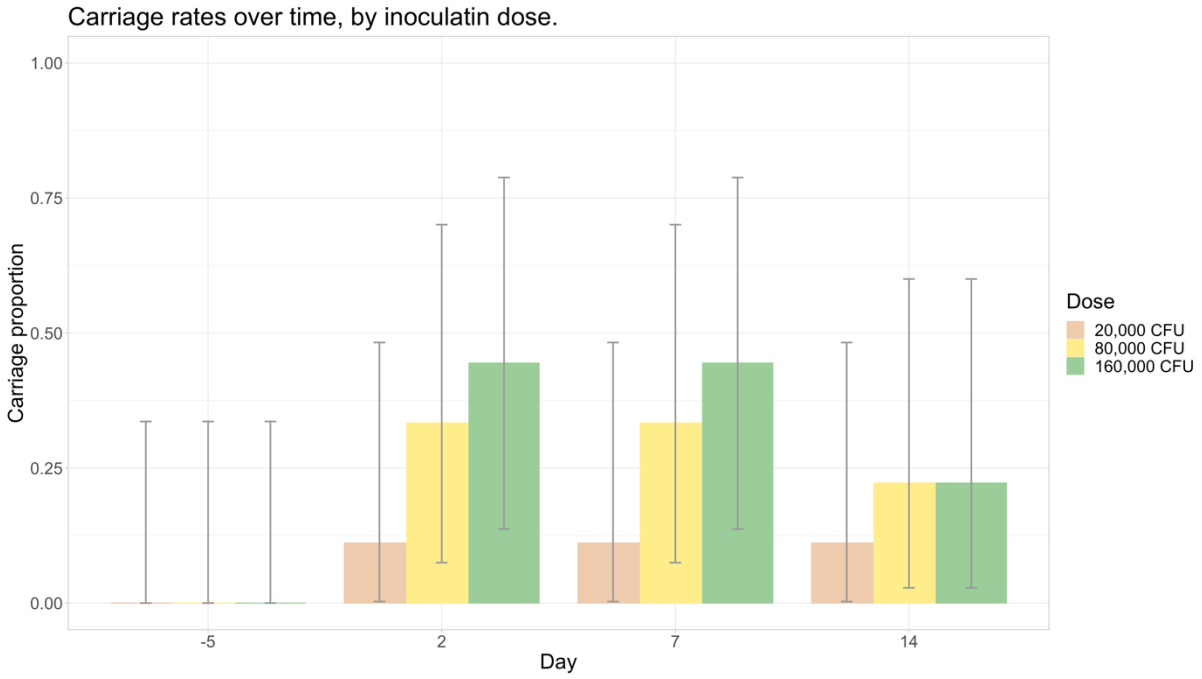

Figure 8.3: Carriage rates over time, by inoculation dose.

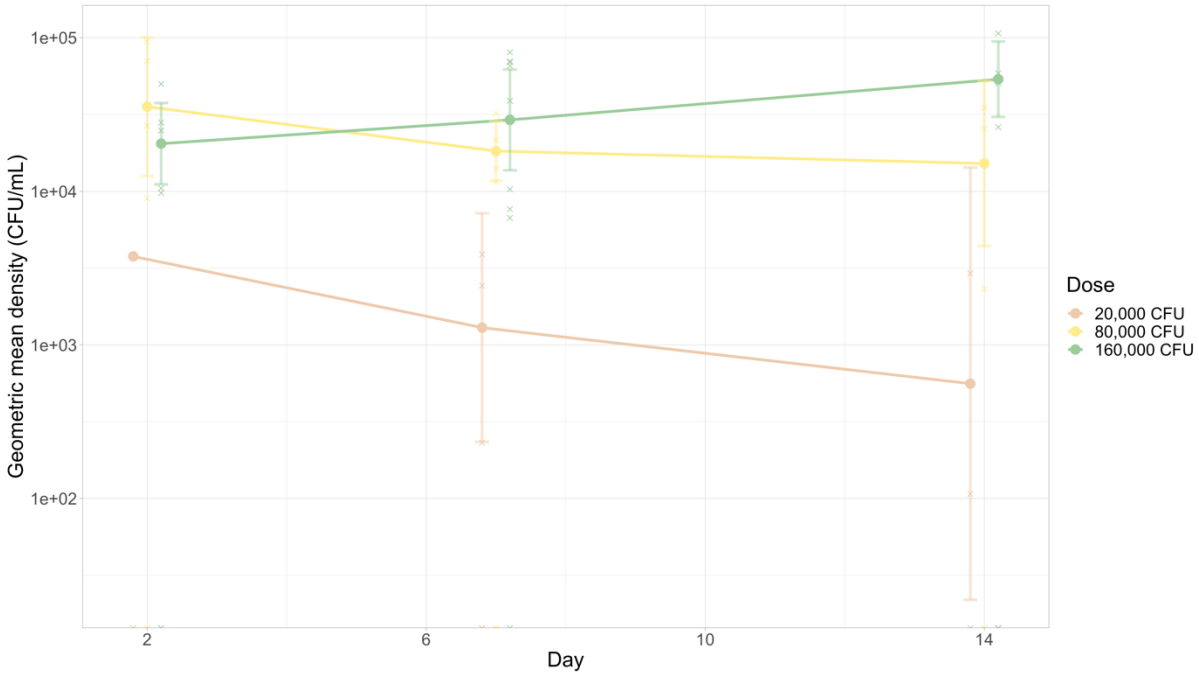

Figure 8.4: Geometric mean carriage density and 95% confidence intervals for colonised participants over time, by inoculation dose. Density was measured at days 2, 7 and 14; values were slightly offset on the x-axis for the different doses for clearer visualisation. Crosses indicate individual data points.

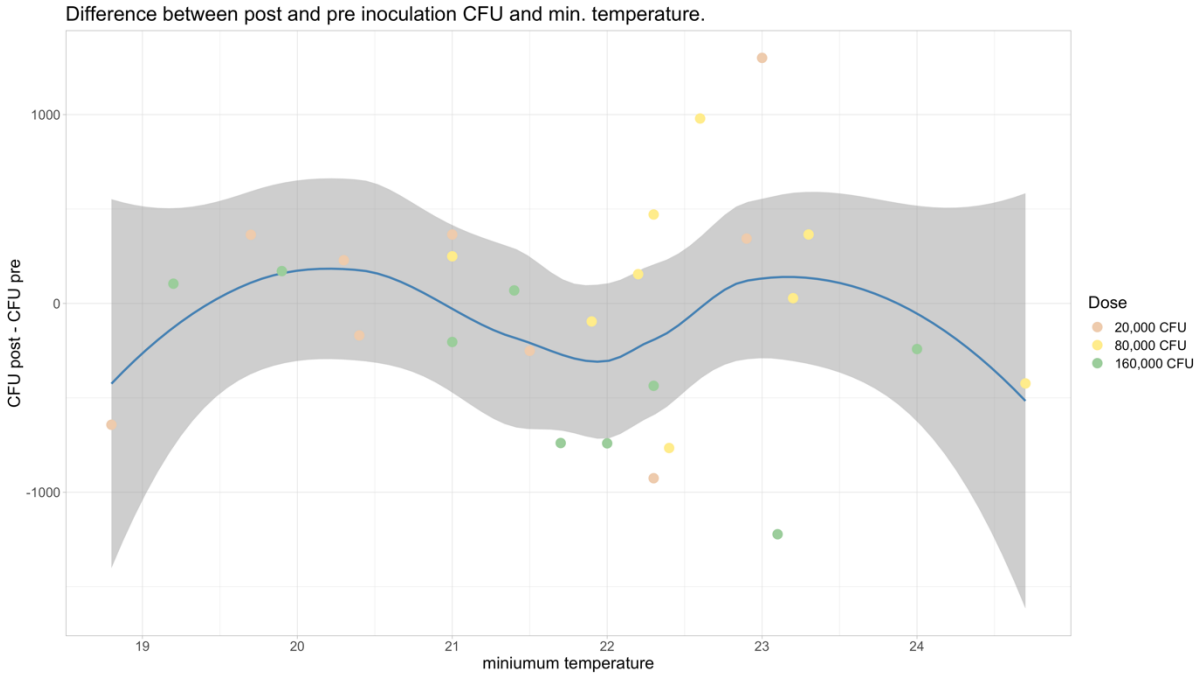

Figure 8.7: Difference in CFU density of the inoculum between pre and post inoculation measurement against minimum recorded temperature.

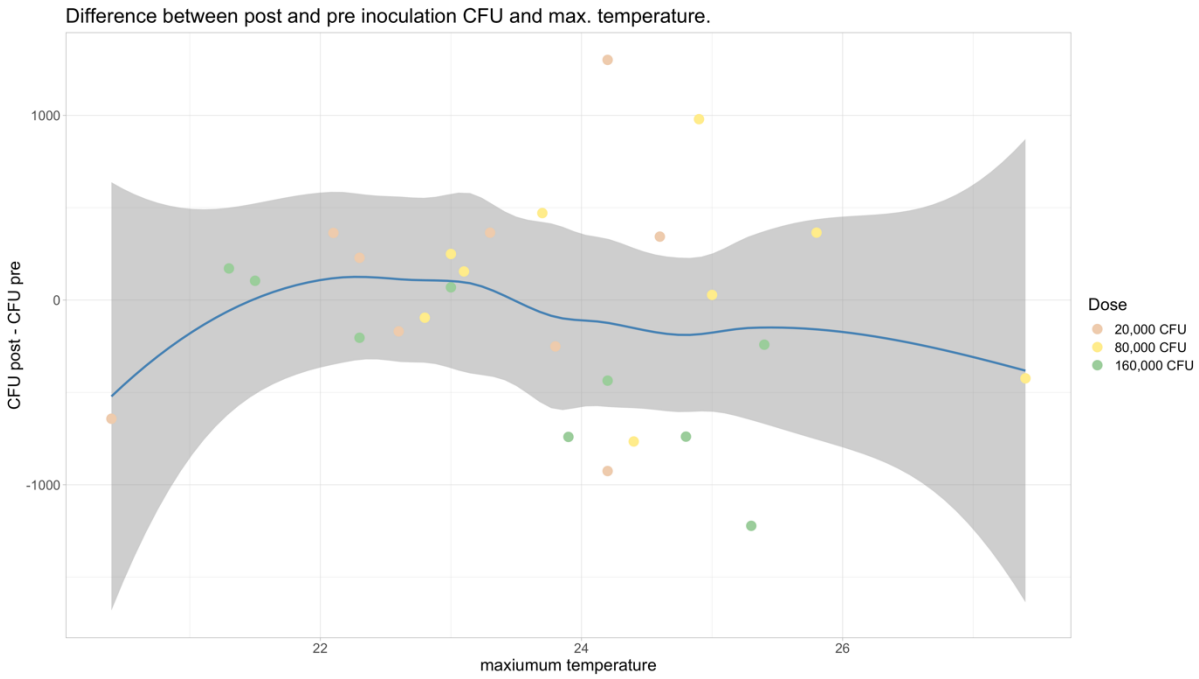

Figure 8.8: Difference in CFU density of the inoculum between pre and post inoculation measurement against maximum recorded temperature.

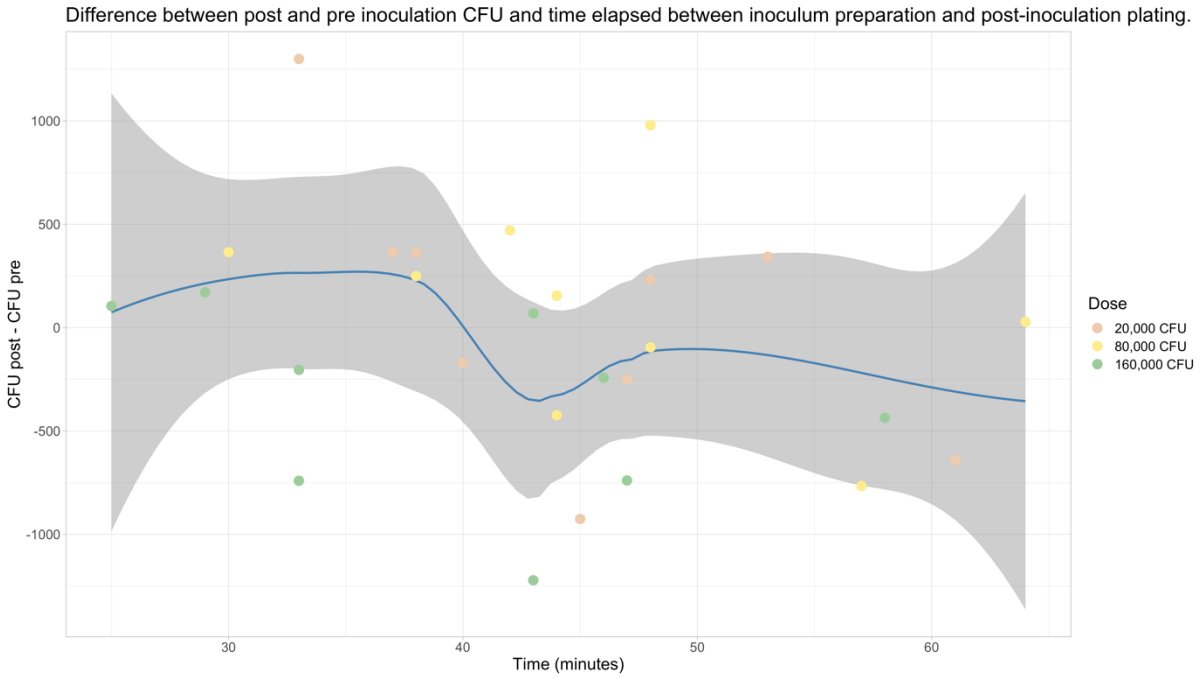

Figure 8.9: Difference in CFU density of the inoculum between pre and post inoculation measurement against time elapse between inoculation preparation and post-inoculation plating.

8.4 Mock Tables and Listings

		Carriage 6B			Carriage non-6B		
	Total	n	Percentage	95% CI	n	Percentage	95% CI
20,000 CFU	9	1	11.1%	(0.3%,48.2%)	1	11.1%	(0.3%,48.2%)
80,000 CFU	9	3	33.3%	(7.5%,70.1%)	0	0.0%	(0.0%,33.6%)
160,000 CFU	9	5	55.6%	(21.2%,86.3%)	1	11.1%	(0.3%,48.2%)
Control	9	0	0.0%	(0.0%,33.6%)	0	0.0%	(0.0%,33.6%)

Table 8.1: Carriage rates for each group.

		Culture		
		negative	positive	NA
Day 2				
lytA qPCR	**negative**	22	6	0
	positive	5	3	0
	NA	0	0	0
Day 7				
lytA qPCR	**negative**	20	2	0
	positive	7	7	0
	NA	0	0	0
Day 14				
lytA qPCR	**negative**	25	3	0
	positive	6	2	0
	NA	0	0	0

Table 8.2: Confusion matrix between culture (columns) and lytA qPCR (rows) carriage detection.

	Visit	Concordant records	Records with both measurements	Concordance	95% CI
All					
	Day 2	25	36	69.40%	(51.9%,83.7%)
	Day 7	26	36	72.20%	(54.8%,85.8%)
	Day 14	28	36	77.80%	(60.8%,89.9%)
20,000 CFU					
	Day 2	8	9	88.90%	(51.8%,99.7%)
	Day 7	8	9	88.90%	(51.8%,99.7%)
	Day 14	8	9	88.90%	(51.8%,99.7%)
80,000 CFU					
	Day 2	3	9	33.30%	(7.5%,70.1%)
	Day 7	5	9	55.60%	(21.2%,86.3%)
	Day 14	7	9	77.80%	(40.0%,97.2%)
160,000 CFU					
	Day 2	5	9	55.60%	(21.2%,86.3%)
	Day 7	4	9	44.40%	(13.7%,78.8%)
	Day 14	4	9	44.40%	(13.7%,78.8%)
Control					
	Day 2	9	9	100.00%	(66.4%,100.0%)
	Day 7	9	9	100.00%	(66.4%,100.0%)
	Day 14	9	9	100.00%	(66.4%,100.0%)

Table 8.3: Concordance proportions for each visit. Concordance has been calculated only for those cases where both measurements were recorded.

	Day 2 (95% CI)		Day 7 (95% CI)		Day 14 (95% CI)	
20,000 CFU	3,768	not enough data	1,295	(233, 7,187)	559	(22, 14,277)
80,000 CFU	35,540	(12,526, 100,838)	18,283	(11,672, 28,640)	15168	(4,399, 52,308)
160,000 CFU	20,417	(11,096, 37,568)	29,173	(13,706, 62,092)	53678	(30,489, 94,502)

Table 8.4: Mean geometric carriage density in colonised participants, stratified by inoculation dose.

		AEs									SAEs	SARs	SUSARs
	n	Total	Sore throat	Coryzal symptoms	Headache	Earache	Feverish	Cough	New rash	Other	Total	Total	Total
All	36	6	1	0	1	0	0	2	2	0	0	0	0
Saline	9	2	1	0	0	0	0	1	0	0	0	0	0
20,000 CFU	9	0	0	0	0	0	0	0	0	0	0	0	0
80,000 CFU	9	0	0	0	0	0	0	0	0	0	0	0	0
160,000 CFU	9	4	0	0	1	0	0	1	2	0	0	0	0
Colonised (6B)	9	2	0	0	0	0	0	1	1	0	0	0	0
Non-colonised	27	4	1	0	1	0	0	1	1	0	0	0	0
Male	17	2	1	0	0	0	0	0	1	0	0	0	0
Female	19	4	0	0	1	0	0	2	1	0	0	0	0

Listing 8.1: Summary of safety data, ovrall and stratified by inoculation group & dose, solonisation status and sex. Total AEs/SAEs/SARs/SUSARs may exceed total number of participants in each group as each participant may report more than one AE/SAE/SAR/SUSAR.

9 References

1. American Statistical Association. Ethical Guidelines for Statistical Practice. (2018).
2. Royal Statistical Society. Code of Conduct. (2014).
3. Collins, A. M. *et al.* First Human Challenge Testing of a Pneumococcal Vaccine. Double-Blind Randomized Controlled Trial. *Am. J. Respir. Crit. Care Med.* **192**, 853–858 (2015).
4. Waddington, C. S. *et al.* An Outpatient, Ambulant-Design, Controlled Human Infection Model Using Escalating Doses of Salmonella Typhi Challenge Delivered in Sodium Bicarbonate Solution. *Clin. Infect. Dis.* **58**, 1230–1240 (2014).
5. Ferreira, D. M. *et al.* Controlled Human Infection and Rechallenge with *Streptococcus pneumoniae* Reveals the Protective Efficacy of Carriage in Healthy Adults. *Am. J. Respir. Crit. Care Med.* **187**, 855–864 (2013).
6. Naclerio, R. M. *et al.* Mediator release after nasal airway challenge with allergen. *Am. Rev. Respir. Dis.* **128**, 597–602 (1983).
7. The Lancet. CONSORT 2010. *The Lancet* **375**, 1136 (2010).
8. Wasserstein, R. L., Schirm, A. L. & Lazar, N. A. Moving to a World Beyond "$p < 0.05$". *Am. Stat.* **73**, 1–19 (2019).
9. R Core Team. *R: A language and environment for statistical computing*. (R Foundation for Statistical Computing, 2018).