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TITLE

Study protocol for Controlled human infection for penicillin G against *Streptococcus pyogenes*: a double blinded, placebo controlled, randomised trial to determine the minimum concentration required to prevent experimental pharyngitis (The CHIPS trial)

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ABSTRACT:

Introduction:

Regular intramuscular benzathine penicillin G injections have been the cornerstone of rheumatic heart disease (RHD) secondary prophylaxis since the 1950s. As the pharmacological correlate of protection remains unknown, it is difficult to recommend changes to this established regimen. Determining the minimum effective penicillin exposure required to prevent *Streptococcus pyogenes* infection will accelerate development of new long-acting penicillins for RHD prevention as well as inform opportunities to improve existing regimens. The CHIPS trial will address this knowledge gap by directly testing protection afforded by different steady state plasma concentrations of penicillin in an established model of experimental human *S. pyogenes* pharyngitis.

Methods and analysis:

This is a double-blinded, placebo-controlled, randomised experimental human infection study. Sixty healthy adult volunteers aged 18 to 40 years will be recruited and randomised 1:1:1:1:1 to continuous intravenous penicillin infusions targeting five different steady state plasma concentrations of 0 (placebo), 3, 6, 12 and 20 ng/mL via a midline catheter. Each participant's penicillin pharmacokinetic parameters will be established prior to the challenge, to ensure accurate dosing for the continuous infusion. Following challenge with a well-characterised strain of *S. pyogenes*, participants will be observed for up to 6 days for development of pharyngitis and treated with antibiotics prior to discharge. The primary objective is to determine the minimum effective steady-state plasma penicillin concentration required to prevent experimental pharyngitis. Secondary objectives will explore systemic and mucosal immuno-inflammatory responses during pharyngitis, bacterial colonisation dynamics, environmental contamination, and qualitative evaluation of the participant experience.

Ethics and dissemination: Ethical approval has been obtained. Findings will be reported in peer reviewed publications and presented at national/international stakeholder forums.

Trial Registration number: ACTRN12621000751875

STRENGTHS AND LIMITATIONS OF THIS STUDY:

1. The minimum effective dose of penicillin to prevent *S. pyogenes* infection is a vital piece of missing information for secondary prevention ARF/ RHD (over 40 million people affected worldwide) and will drive innovation of better penicillin formulations.
2. This is the first human challenge infection study to evaluate the minimum concentration of an antimicrobial required to prevent infection.
3. Bias will be reduced by randomising participants to placebo, or different target plasma concentrations between 3 and 20 ng/mL using individualised data from a single dose pharmacokinetic study conducted prior to the challenge.
4. The challenge *S. pyogenes* strain has been selected after extensive characterisation which has a limited virulence profile and a well-defined and repeatable minimum inhibitory concentration (12 ng/mL).

INTRODUCTION

Recurrent infections with *Streptococcus pyogenes* (Group A beta-haemolytic Streptococcus; *S. pyogenes*) are associated with development of acute rheumatic fever (ARF) and rheumatic heart disease (RHD).[1] RHD affects 40.5 million people globally and causes 306,000 deaths annually, mostly children and young adults living in low- and middle-income countries.[2, 3] The efficacy of 3- to 4-weekly intramuscular (IM) injections of 1.2 million units (MU; 900 mg) benzathine benzylpenicillin G (BPG) for RHD secondary prophylaxis was first demonstrated in the 1950s and remains the only proven and cost-effective protection against recurrent infection and progressive RHD.[4-6] After deep IM injection, BPG is slowly hydrolysed to benzylpenicillin G (penicillin) and absorbed into the plasma.[7]

While secondary prophylaxis has been shown to be moderately effective in adherent individuals, poor adherence to painful monthly intramuscular injections (recommended for a minimum of 10 years) limits coverage of secondary prophylaxis and its overall effectiveness.[6, 8, 9] There is an urgent need to improve penicillin formulations for patients with ARF and RHD. Stakeholder consultations with consumers and RHD experts have consistently identified the ideal characteristics for secondary prophylaxis which include reducing dose frequency (ideally every 3-6 months), reducing pain of administration, alternative delivery strategies (including injectable implants or non-injection methods) and cold chain independence as key aspirations for an acceptable product.[8, 10] However, as the pharmacological correlate of protection remains unknown, it is difficult to recommend changes to the established regimen.

It has conventionally been assumed that critical pharmacological correlate for prevention of *S. pyogenes* infections is the time between IM injections that plasma penicillin concentrations remain above 0.02 mg/L (20 ng/mL), a typical minimum inhibitory concentration (MIC) for *S. pyogenes* isolates.[11] However, emerging evidence from a number of high risk settings demonstrates that even the most adherent patients do not maintain these target concentrations for the majority of the interval between BPG

injections.[12, 13] Given the apparent efficacy in adherent patients, it is possible that current regimens of BPG confer protection at lower, sustained inter-injection levels of plasma penicillin. Alternatively, transient peaks in serum concentration may be sufficient as an intermittent presumptive treatment.

The opportunity to directly test the former hypothesis under the necessary controlled conditions has arisen with the advent of a new experimental human infection model of *S. pyogenes* pharyngitis in healthy adults.[14] The CHIPS trial will address a key knowledge gap by directly testing protection against experimental human pharyngitis, in relation to different steady state plasma concentrations of penicillin, to inform strategies for pharmacological secondary prophylaxis of RHD, including development of new and more effective long-acting penicillin formulations or optimising dose and dosing intervals with currently available formulations.

METHODS AND ANALYSIS:

Study Design

The CHIPS trial is a double-blinded, placebo-controlled, randomised human infection study to determine the minimum effective steady-state plasma penicillin concentration required to prevent pharyngitis following direct application of *S. pyogenes* to the oropharynx (Figure 1). Based on the successful human challenge model developed in the CHIVAS-M75 study,[14] healthy adult volunteers will be recruited through a private contract research organisation (CRO) and inoculated with the same *S. pyogenes emm*75 strain. A total of 60 participants will be recruited in 4 cohorts of 15 volunteers. Participants will be randomised 1:1:1:1:1 to receive continuous IV infusions of penicillin at 5 possible steady state plasma concentrations of 0 (placebo), 3, 6, 12 and 20 ng/mL. The study will be conducted within the CRO facility in Perth, Western Australia, with clinical support from a nearby tertiary hospital.

Patient and public involvement

The need to improve RHD secondary prophylaxis is underpinned by extensive consumer engagement which has consistently identified pain and frequency associated with BPG injections as barriers to adherence.[8, 15, 16] However, as this is a human infection study involving healthy volunteers, there is limited scope for health consumer input into the design and implementation of the study. While the methodology of our study involves *S. pyogenes* pharyngitis and its prevention using continuous penicillin infusion, the focus of our research and its intended beneficiaries are not sufferers of pharyngitis, but rather secondary prophylaxis for those living with ARF/RHD. Due to this indirect connection, involvement of target patient population would be premature. For the healthy volunteers who participate in the study, they will be made aware of results of this trial and informed of how to access the published findings.

Study objectives and outcomes

The primary objective is to determine the minimum plasma penicillin concentration associated with protection against experimental *S. pyogenes* pharyngitis following the challenge, assessed by the development of acute symptomatic pharyngitis (primary outcome) during the confinement period. This is assessed using the pharyngitis case definition from CHIVAS-M75 study, incorporating elements of clinical prediction rules based on Centor and McIsaac scores, change in tonsil size and real time molecular point-of-care test for *S. pyogenes* (ID NOW Strep A2, Abbott).[14] Secondary objectives include identification of plasma penicillin concentration required to prevent pharyngeal colonisation of *S. pyogenes*, and characterisation of immune responses and inflammatory profiles comparing participants across penicillin dose bands and pharyngitis outcome. Exploratory objectives are to examine *S. pyogenes* potential for environmental contamination (with relevance to disease transmission) and explore motivations and the lived experiences of the volunteers who take part in human infection studies (listed in Table 1 along with outcome/endpoint assessments).

Table 1: Study objectives and outcomes

	Objective(s)	Outcome(s)/ Endpoint(s)
Primary	To determine the minimum plasma penicillin concentration required to prevent acute symptomatic *S. pyogenes* pharyngitis following a direct oropharyngeal challenge with *S. pyogenes* M75	Development of *S. pyogenes* pharyngitis during confinement period, according to a pre-defined clinical and laboratory criteria
Secondary	1. To identify the target plasma penicillin concentration required to prevent *S. pyogenes* colonisation of the pharynx	Development of *S. pyogenes* colonisation following challenge, defined as *S. pyogenes* M75 isolation from throat swab in absence of signs and symptoms of clinical pharyngitis after completing antibiotic treatment at conclusion of confinement period.
	2. To identify the target salivary penicillin concentration required to prevent *S. pyogenes* pharyngitis or colonisation	Assays to detect penicillin concentration in saliva from all participants
	3. To characterise plasma humoral and cellular immunological profiles of immune response to experimental challenge with *S. pyogenes* in healthy participants	Laboratory assays to measure immunological and inflammatory responses to the challenge
	4. To characterise plasma inflammatory (CRP and procalcitonin) and metabolomic profiles of *S. pyogenes* pharyngitis	Measurement of inflammatory markers from blood samples
	5. To identify whether Cystatin C- based markers of renal function improve estimates of penicillin G renal clearance compared with creatinine-based measures	Measurement of Cystatin-C from blood samples
	6. To explore microbiological and local factors associated with *S. pyogenes* adhesion to tonsillar mucosa	Laboratory assays to measure mucosal response
	7. To explore microbiological and local factors associated with *S. pyogenes* adhesion to tonsillar mucosa	Laboratory assays to measure mucosal response

	8. To explore *S. pyogenes* transcriptomic changes in response to penicillin exposure in *S. pyogenes* pharyngitis	Laboratory assays to measure mucosal response
	9. To investigate potential environmental contamination of *S. pyogenes* via large respiratory droplets, airborne small respiratory droplets, and surface contact	Microbiological and culture analysis of participants' contact surfaces and surroundings
	10. To explore motivations, attitudes, and experiences of participating in clinical trials and human challenge studies	Reponses to questionnaires administered during study period by participants

Recruitment and eligibility

A database of healthy volunteers maintained by the CRO will be used for recruitment of study participants, along with multi-media advertisements (e.g., social media platforms of CRO and affiliated institutions) using materials approved by the ethics committee. Participants will be financially reimbursed of a value determined to be satisfactory by the ethics committee. Healthy adult males and non-pregnant, non-lactating females aged 18 – 40 years without pre-existing risk factors for severe *S. pyogenes* disease will be recruited. Strict eligibility criteria are in place to mitigate risks to potential participants (detailed in Supplementary Materials 1). In addition to usual 'healthy adult' inclusion and exclusion criteria, medical history and physical examination, prospective participants will undertake electrocardiography and transthoracic echocardiography to rule out undiagnosed sub-clinical cardiac pathology. They will also undergo screening throat swabs and a serum *emm*75 type-specific serology to exclude carriage and prior immunity to *S. pyogenes emm*75 strains, respectively.

Study Interventions

Dose-finding pharmacokinetic study

The overall journey of a participant from screening to completion of follow up is illustrated in Figure 2. At least several days prior to the inpatient challenge admission, each participant will have an individual pharmacokinetic dose-finding assessment. A 600 mg bolus dose of intravenous penicillin will be administered and serial venous blood samples will be collected for plasma penicillin concentration measurements at baseline, then 15, 30, 60, 120, 180, 240, and 360 minutes afterwards. Clearance and volume of distribution will be derived to enable calculation of individualised IV penicillin continuous infusion doses to attain the randomised target concentration for the *S. pyogenes* challenge admission.

Randomisation procedure

First 45 participants will be randomised 1:1:1:1:1 to one of 5 different target steady state concentrations (0 [placebo], 3, 6, 12 and 20 ng/mL). To ensure there is at least one participant in each concentration for each group of 5 and 3-per-concentration in each cohort of 15 participants, the volunteers will be block randomised in groups of 5 following an allocation sequence generated by the study statistician and stored on a secure server, accessible only to the unblinded pharmacy and analytical team members. All clinical staff and participants will remain blinded to the treatment allocation (concentration level) for the duration of the study.

Challenge procedures

Participants are considered enrolled from the time of commencing the penicillin infusion via a midline intravenous catheter on the day of admission (Day -1). On the day of challenge (Day +1), a sterile Dacron swab is dipped in a 1mL single-dose vial containing 1-3 x 10^5 colony-forming units (CFU) of *emm*75 *S. pyogenes* and applied directly to the participant's oropharynx. The single-dose vials will be produced according to the principles of Good Manufacturing Practice.[17, 18] Each participant will be challenged once only, using a standardised procedure analogous to a diagnostic throat swab done 'in reverse', as previously described.[17] Participants will be fasted for 90 minutes before and after challenge.

Confinement and discharge

Following challenge, participants will remain inpatients at the CRO facility until reaching the primary pharyngitis outcome or until 5 days after challenge if they remain asymptomatic, whichever occurs first. The penicillin infusion will stop at that time and a separate oral antibiotic course will be initiated (azithromycin 500 mg once daily for 5 days[19]). All participants will be monitored as inpatients for at least 24 hours after their first dose of oral antibiotic prior to discharge. Subsequent safety follow-up visits will occur 7 and 28 days after the first dose of oral antibiotic. Participants will return unused antibiotic tablets which will allow monitoring of adherence to the remainder of oral treatment.

Adding/removing treatment arms

After 45 participants (3 cohorts), an interim analysis will be performed. Provided that the pre-specified statistical thresholds are met, the investigators may adjust the target concentration arms (while retaining the placebo arm) for the last cohort of 15 participants (to concentrations up to 100 ng/mL) to increase the precision of the minimum effective concentration estimate.

Pharmaceutical handling of penicillin

For each participant, individual pharmacokinetic parameters will inform the penicillin dose required to prepare the intravenous infusion bags for all possible dose allocations. After randomisation, infusion bags will be prepared at an aseptic compounding facility according to the individualised calculated dose to attain the allocated steady state plasma penicillin concentration. The stability of benzylpenicillin in 0.9% w/v sodium chloride IV infusion bags and the optimum sodium citrate concentration has been formally evaluated for the CHIPS trial (manuscript accepted[20]). These stability studies demonstrated excellent chemical preservation of buffered benzylpenicillin at room temperature, with <1% degradation after 24 hours for benzylpenicillin 25 µg/mL in sodium citrate 100 µg/mL in 0.9% w/v sodium chloride solution, whether exposed to or protected from artificial light. Continuous infusion bags will be routinely changed

every 24 hours. A sample of remnant fluid from each bag will be collected, stored at -80 °C, and assayed to confirm the expected stability of penicillin over the 24-hour period.

Measurement of possible environmental contamination and transmission potential

At 3 post-challenge timepoints (+24, +36 and +48 hours), Colistin Nalidixic Acid (CNA) agar plates will be placed in the participant's room for 4 hours to capture potential droplet or airborne transmission of *S. pyogenes*. Swabs will also be taken of the participant's surroundings and personal devices (approximately 25cm^2). To detect droplet transmission potential, participants will read a short text at each timepoint with CNA plates placed at varying lengths (30cm, 90cm and 180cm). All swabs and CNA plates collected will undergo transfer and processing for microbial culture for beta-haemolytic Streptococci (BHS) as per Clinical and Laboratory Standards Institute (CLSI) standards, including use of positive and negative controls. If no growth is detected after 24 hours, incubation will be extended for another 24 hours. Presence of *S. pyogenes* from beta-haemolytic colonies will be confirmed with agglutination kits using group specific antigens (Streptex™, Thermo Scientific).

Study participants' experience

Participants will complete surveys at 3 timepoints: admission, at diagnosis of pharyngitis or Day +3 (if asymptomatic), and immediately prior to discharge. These surveys will collect qualitative data using standardised questionnaires evaluating participant's motivation for involvement in the study and how expectations or concerns held prior to admission compared to the experience. In addition, participants will also be asked to keep a diary and record elements of their challenge admission specific to their experience of participating in a human challenge study in a non-structured form. This qualitative data will be collected and collated into themes for analysis and reporting.

Governance

A unique governance structure, incorporating a Safety Data Review Team (SDRT), has been set up to meet the needs of this study as illustrated in Figure 3. Day-to-day conduct of the study and reporting of its progress to the SDRT is done by the trial management group whose members will remain blinded to the randomised allocation until after completion of study. SDRT has voting members (chaired by an independent expert) who make decisions regarding continuation of trial after each cohort of 15 is completed, with non-voting members from the analytical team and trial steering committee providing an advisory role. An independent study monitor will be engaged who will ensure that the investigation is conducted according to the protocol and regulatory requirements. Strict data management plan will be adhered to protect participant confidentiality in compliance with Good Clinical Practice guidelines.

Safety

As in the CHIVAS-M75[14] study, the following will be considered medically significant events in addition to the standard definitions – local and systemic complications of *S. pyogenes* infection, autoimmune sequelae of *S. pyogenes* infection (such as ARF, RHD, and glomerulonephritis), recurrent pharyngitis in participants caused by the challenge strain, and secondary cases of *S. pyogenes* infection with the challenge strain in non-participants.

Participant safety during confinement: Participants will be monitored closely during the confinement period in a purpose-built clinical trials facility with 24-hours clinical staffing and twice daily reviews. All adverse events will be recorded in real time and any serious adverse event (SAE) will be reported to the SDRT within 24 hours of their occurrence. Starting a new cohort of participants will require approval by the SDRT following an interim review after each cohort's confinement period is completed.

Long term safety: The risk of long-term carriage of *S. pyogenes* is minimised with treatment using a non-beta-lactam antibiotic (azithromycin) prior to discharge. Additional reassurance comes from the CHIVAS-

M75 study in which none of 25 participants challenged with the *emm*75 strain had developed persistent carriage, systemic or autoimmune complications of *S. pyogenes* at completion of 6 months follow up.[14] The risk of secondary spread of infection from participants will be negligible as they will be confined in a clinical trial facility with stringent infection control measures and will have had 24 hours of oral antibiotic treatment by the time of discharge back to community (in keeping with public health recommendations for school exclusion).

Challenge strain

The *S. pyogenes* challenge strain (*emm*75, M75) was isolated from a patient with pharyngitis in 2011. It has been extensively characterised and selected for its suitability for human challenge. It is an infrequent *emm*-type in most published series but reliably causes pharyngitis. The particular challenge strain has favourable antibiotic susceptibility, and does not have a virulence profile typical of hypervirulent strains.[18] The characterisation, selection, manufacture, storage, and quality assurance approach for the *emm*75 challenge strain *S. pyogenes* has previously been described.[14, 17] In the CHIVAS-M75 study, at the starting dose level of 1-3 x 10^5 CFU in each single-dose vial, the pharyngitis attack rate was 85%.[14]

Sample size calculation

Based on simulations, a maximum of 60 participants are required (recruited in 4 cohorts of equal size; starting with 5 treatment arms) to detect a minimum effective dose (MED) between 0-20 ng/mL, with >80% power and Type 1 error <5%. Trial simulations were based on: (i) an anticipated 25% of placebo participants symptom-free at the end of study Day +5; (ii) a monotonic normal dynamic linear model with weakly informative prior distributions; (iii) equal allocation to all treatment arms; (iv) interim analyses after each cohort has completed (i.e. every 15 participants); (v) a high target of 90% symptom-free and a low target of 80% symptom-free in determining the MED; and (vi) stopping rules for success if the posterior probability that the MED is greater than the low target is greater than 80% (i.e. pr(MED>low

target)>80%) and for futility if the posterior probability that the MED is greater than the upper target is less than 10% (i.e. pr(MED>upper target)<10%). Trial operating characteristics were calculated for 8 scenarios ranging from null efficacy to MED detected at the highest dose level.

Data analysis plan

Study data will be collected using paper and electronic source documents and managed using a secure institution hosted electronic database (Research Electronic Data Capture, USA). For the primary endpoint, Bayesian analyses will be performed on the accumulating data after each cohort completes study Day +5 and the primary pharyngitis endpoint is determined for each participant. It is anticipated that up to 25% of participants in the placebo arm may remain free from pharyngitis. A monotonic normal dynamic linear model will be used to assess the dose response and estimate the MED. After the completion of the second (n=30) and third (n=45) cohorts, we will evaluate stopping rules for success (pr(MED>low target)>80%) and for futility (pr(MED>upper target)<10%). All secondary outcomes will be summarised by treatment arm using appropriate statistics, including mean and standard deviation for continuous variables with symmetrical distributions or median and interquartile range for asymmetric distributions. Categorical variables will be summarised using frequencies and percentages.

ETHICS AND DISSEMINATION

This protocol (Universal Trial Number U1111-1264-9535) has been reviewed and approved by the Bellberry Human Research Ethics Committee (Ref: 2021-03-295) and is registered on the Australian New Zealand Clinical Trials Registry (www.anzctr.org.au, ACTRN12621000751875). The sponsor is the Telethon Kids Institute and the study is indemnified under the existing institutional insurance policy. The results of the study will be of national and international significance. Our team has strong links to stakeholder groups and national and international profiles that will ensure dissemination of the results in peer-reviewed journals and presentation at relevant congresses.

DISCUSSION

The Global Resolution on Rheumatic Fever and Rheumatic Heart Disease calls for new technological approaches to improving global RHD control, including the "development of a long-acting formulation of penicillin that might improve secondary prophylactic regimens".[21] The CHIPS trial aims to address a key knowledge gap toward achieving this goal, by challenging the dogma regarding what is the MED of penicillin for successful prevention of *S. pyogenes* infection and secondary RHD prophylaxis.

Experimental human infection, or challenge studies, have been core platforms for infectious diseases research since at least Edward Jenner's 18th century smallpox vaccine studies.[22] In recent decades, reinforced by rigorous ethical frameworks and independent safety reviews, modern human challenge trials have contributed to accelerating development of new drugs, vaccines, and diagnostics.[22, 23] The CHIPS trial is the first to take advantage of the successful establishment of a human pharyngitis model in the CHIVAS-M75 study.[14] Although human challenge trials have previously tested the efficacy of drugs, the use of randomised steady-state plasma concentration levels of penicillin aiming to demonstrate a prophylactic threshold is an innovative approach among modern human challenge trials.[22-24] Even with a sample size of 60 participants, our study is adequately powered to detect the anticipated effect size.[25-28] The minimum effective dose of penicillin established in the present study is expected to provide evidence that sustained low plasma penicillin concentrations (<20 ng/mL) do prevent *S. pyogenes* infection while shedding further light on the relationship between serum and tissue penicillin concentrations and the role they might play in host-pathogen interaction leading to pharyngitis. Pharmacokinetic correlates of protection identified will inform the target product profile for long-acting penicillin formulations and the development of implants or depot injections that provide a 'smoother' exposure profile to overcome the need for frequent painful injections associated with low adherence rates.[6, 8, 29]

Findings from the CHIPS trial may have wider applications beyond RHD secondary prophylaxis, for other indications for penicillin prophylaxis, such as recurrent lower extremity cellulitis. *S. pyogenes* is a leading cause of erysipelas and cellulitis, the latter commonly affecting older adults living in developed economies.[30-33] In the United States alone, it is estimated to cost $3.7 billion in healthcare expenditure from 14.5 million cases annually.[34] In addition, improved long-acting formulations of penicillin could have advantages for treatment of certain conditions including syphilis and other treponemal syndromes.

Potential limitations of our approach include the uncertain generalisability of a MED related to a single strain. Nonetheless, notwithstanding recent concerns stemming from rare penicillin binding protein mutations, *S. pyogenes* remains universally susceptible to penicillin.[35-37] The relationship between *in vitro* MIC of the *emm*75 challenge strain and the MED determined in the CHIPS trial will still be relevant to considering other strains with different MICs. Likewise, it is uncertain whether findings in healthy volunteers are generalisable to the relevant patient groups for RHD secondary prophylaxis. Certainly, future research will be needed to validate novel regimens of existing preparations or novel formulations which are underpinned by new knowledge obtained from this trial across a broad range of target patient populations. The CHIPS trial will also be a platform to build on insights into host-pathogen interactions already emerging from the CHIVAS-M75 trial, to exploring environmental determinants of transmission, and adding to the literature exploring the experience of participants in human challenge trials.[38, 39]

AUTHORS' CONTRIBUTIONS

TKH, JO and LM prepared the first draft of the manuscript. JO, LM and JC conceived the study design. All authors contributed to the development of the study protocol and revision of the manuscript and have approved the final manuscript.

COMPETING INTERESTS STATEMENT

Investigators for this study have no financial or other competing interests.

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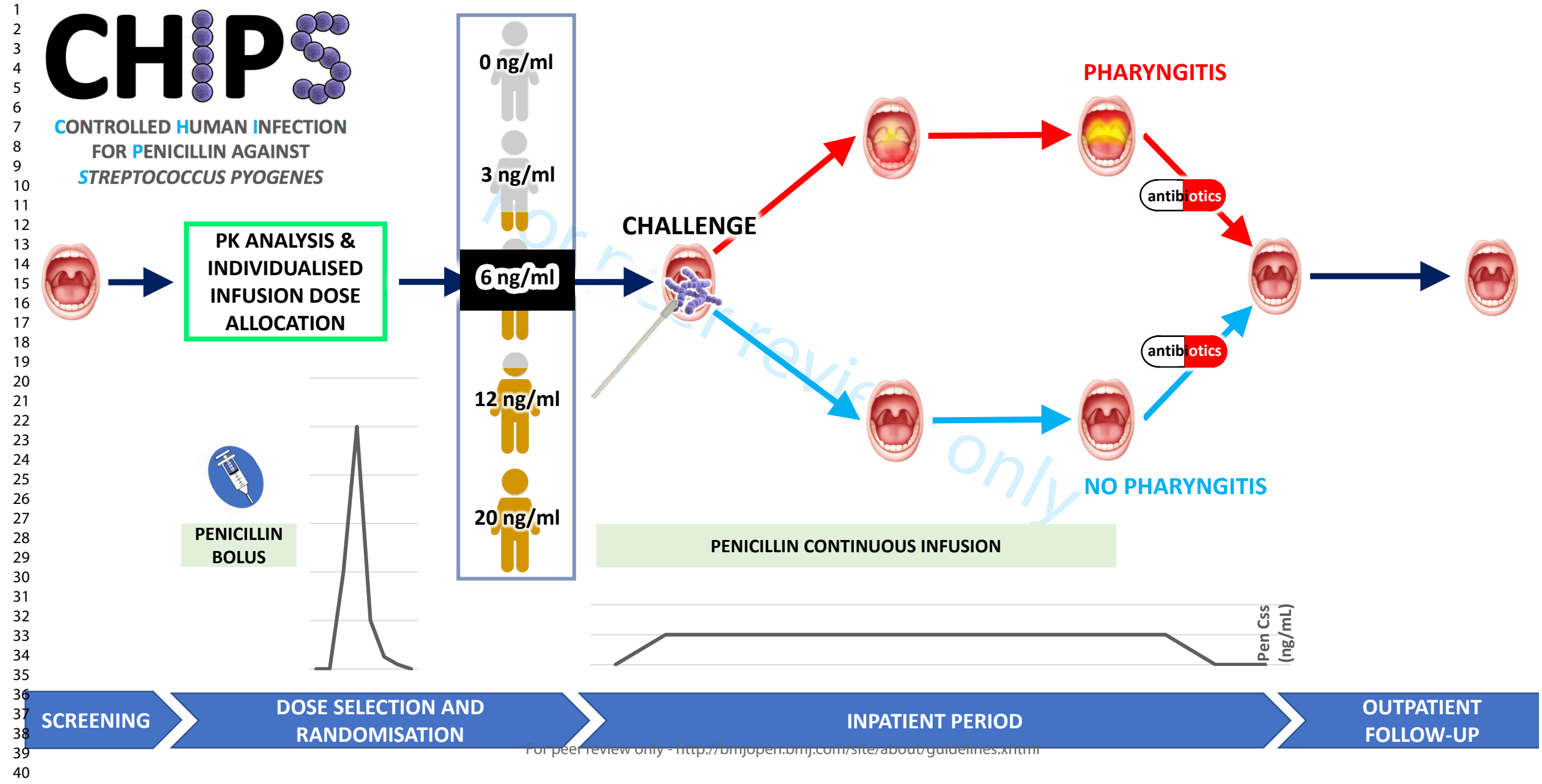
FIGURES

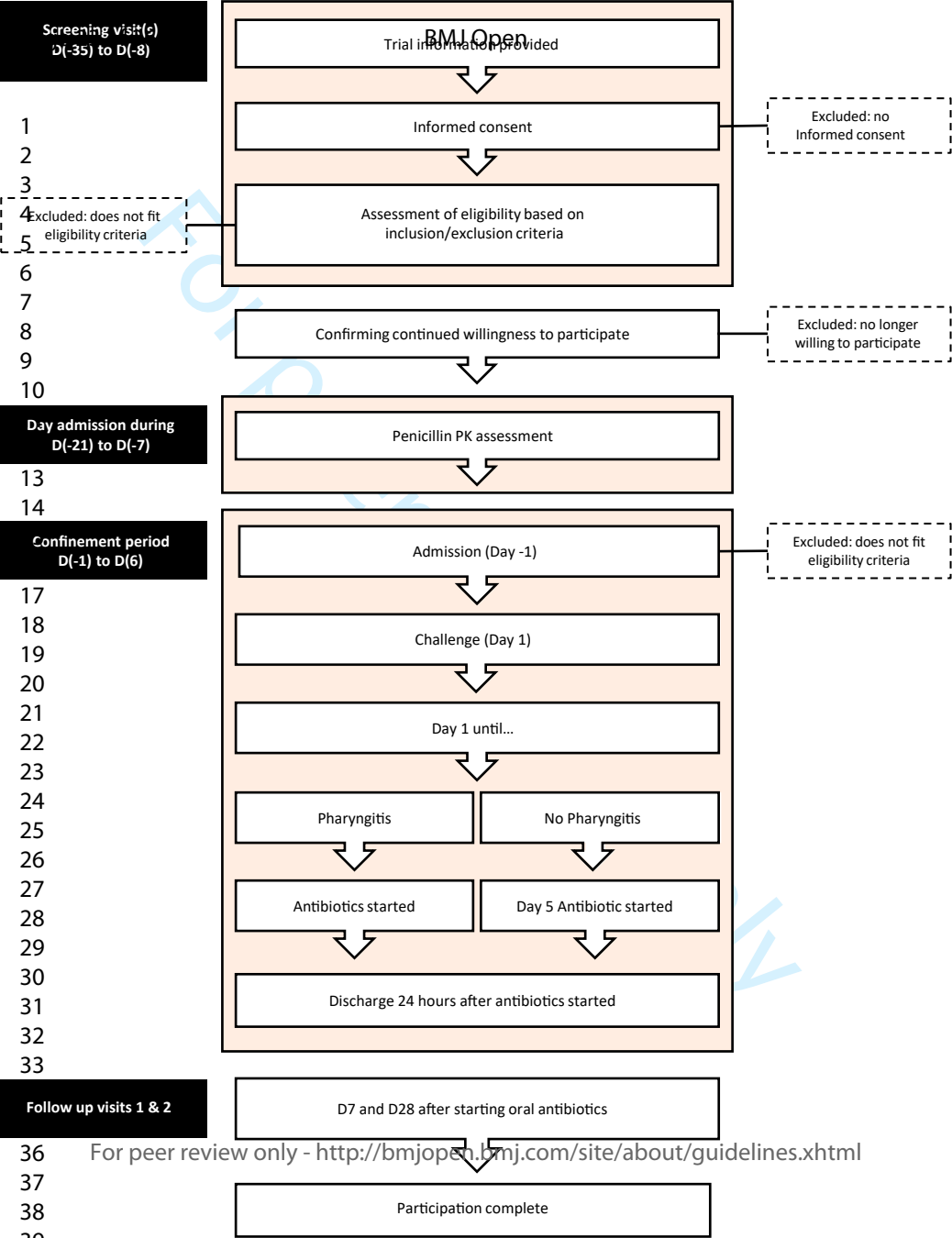
Figure 1: Pictorial synopsis of the CHIPS study. (Pen Css, penicillin steady state concentration; PK, pharmacokinetic)

Figure 2: Participants' journey through the CHIPS study.

Figure 3: Schematic illustration of governance structure and information flow. (AE – adverse event; CRO – contract research organisation; LCMS – liquid crystallography mass spectrometry; PI – principal investigator; PICC – peripherally inserted central catheter; SAE – serious adverse event)

WORD COUNT - 3406





Trial Management Group

Day to day delivery & conduct of the trial:

- Not independent of trial
- Remains blinded to participant allocation, efficacy and safety reports for the entire trial

Comprising:

PI's delegate, PICC nurses, CRO Nursing/Medical Staff

Continue Trial: Yes/No

SAE/AE Reports

Primary outcome assessments

Individualised penicillin doses

Logistical issues

Safety Data Review Team (SDRT)

Voting members (mandatory attendance) comprising:

- **Independent medical monitor (Infectious Diseases Expert and SDRT Chair – preferably with experience in human challenge models)**
- **Independent *S. pyogenes* expert**
- **Independent trial statistician**
- **A/Prof Laurens Manning (PI)**

- Receives reports on SAE and AE with recommendations
- Provides recommendation to continue trial after each cohort of 15 participants
- Provides recommendation to modify treatment allocations with interim analyses with advice from non-voting team members

Non-voting members comprising:

Analytical Team (mandatory attendance):

Pharmacometrician, trial biostatistician, LCMS analyst

- Not-independent of trial
- Responsible for treatment allocation, individualised dosing & planned interim analyses
- Provides advice to voting members

Trial steering committee (invitees, as required attendance):

Listed CHIPS Investigators

- Not independent of trial
- Executive advice to voting members which considers the interests of trial, participants, funder, sponsor

SUPPLEMENTARY MATERIAL

Inclusion and exclusion criteria for eligibility to participate in the CHIPS study

Inclusion criteria
1. Males or females, aged 18 - 40 years (inclusive).
2. Body mass index of 18.0 - 32.0 kg/m^2, inclusive, and body weight ≥ 50.0kg
3. Medically healthy, determined by medical history, physical examination, transthoracic echocardiogram, non-clinically significant laboratory profiles, vital signs, and 12-lead ECG at screening.
4. Systolic blood pressure (SBP) of 90 mmHg – 140 mmHg and diastolic blood pressure (DBP) of 40 mmHg - 90 mmHg.
5. Resting heart rate (HR) of 40-100 bpm
6. Females must be non-pregnant, non-lactating or postmenopausal.
7. Females of childbearing potential must agree to use a barrier method for the duration of study
8. Must be willing and able to read, understand, and sign the participant information and consent form. Willing to comply with all study requirements.
9. Willing to abstain from the use of mouthwash from the day of screening until the first outpatient visit.
10. Must be willing for insertion and have adequate sites for placement of indwelling intravenous cannulae and midline intravenous catheter.

Exclusion criteria
1. Evidence of pre-existing immunity to the challenge strain, defined for this study as a high serum IgG to a peptide comprising the first fifty amino acids of the M75 protein (N-terminal hypervariable region) measured by ELISA. 2. Currently taking penicillins or use of any penicillin-based antibiotics from screening through to the final study visit. The use of probenecid, NSAIDs, or other medications which may significantly alter penicillin PK will also not be permitted within 14 days prior to study drug administration until completion of the final follow-up visit. 3. Any corticosteroid, immunomodulator or anticoagulant use in the previous 3 months, antiplatelet use in the previous 2 weeks, or anticipated use of such drugs during the study period. Any participant currently receiving or having previously received immunosuppressive therapy, including systemic steroids including adrenocorticotrophic hormone (ACTH) or inhaled steroids in dosages which are associated with hypothalamic-pituitary-adrenal axis suppression such as 1 mg/kg/day of prednisone (or its equivalent) or chronic use of inhaled high potency corticosteroids (budesonide 800 µg or fluticasone 750 µg per day). Intranasal corticosteroid use is not allowed from 14 days prior to admission, during the confinement period, and is discouraged prior to the first outpatient visit. Topical corticosteroid use is allowed. 4. Use of prescription or non-prescription drugs (except for oral contraceptive pill in healthy adult females) and herbal supplements (such as St John´s Wort) within 14 days or 5 half-lives (whichever is the longer) prior to the inoculation administration. 5. History of any clinically important cardiac, endocrine, haematological, hepatic, immunologic, metabolic, urologic, pulmonary, neurologic, dermatologic, psychiatric, and renal, or other major disease.

6. History of hospitalisation for illness within the six months prior to enrolment into study, or major surgery within the 12 months prior to enrolment into study.
7. History of tonsillectomy, adenoidectomy or splenectomy.
8. Known or suspected autoimmune disease or impairment/alteration of immune function resulting from:
    a. Congenital or acquired immunodeficiency (including IgA deficiency)
    b. Receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within the preceding 6 months.
9. History of a severe drug reaction or severe allergic reaction (eg. anaphylaxis, convulsions) or clinically significant allergic disease diagnosed by a Physician.
10. Personal or family history of severe S. pyogenes infection or sequelae (such as acute rheumatic fever, rheumatic heart disease, post-streptococcal glomerulonephritis) or invasive S. pyogenes disease (toxic shock syndrome, necrotizing fasciitis, bloodstream infection, pleural empyema, meningitis, puerperal sepsis).
11. Clinically significant disease or any condition or disease that might affect drug absorption, distribution, or excretion, e.g. gastrectomy, diarrhoea.
12. Any vaccination within the last 28 days (except for COVID-19 or seasonal influenza) or use of any antibiotic therapy during the 14 days before challenge.
13. Presence of an acute infectious disease or febrile illness (e.g., sub-lingual temperature ≥ 37.5°C) within the five days prior to challenge with S. pyogenes M75.
14. Significant acute or chronic infection within 14 days prior to inoculation that the Investigator deems may compromise participant safety.
15. Any clinically significant abnormal finding on biochemistry or haematology blood tests, urine analysis, ECG or transthoracic echocardiogram at screening.

16. Laboratory tests that fail to meet the following thresholds:
    a. Haematology: Haemoglobin, haematocrit, red cell count, white cell count with differentials, platelet count, MCH, MCV, MCHC– parameters within gender-specific reference intervals from the local laboratory unless deemed not clinically significant by the investigator.
    b. Clinical chemistry within gender-specific reference intervals from the local laboratory unless deemed not clinically significant by the investigator: urea, glucose, creatinine, sodium, potassium, chloride and bicarbonate, lactate dehydrogenase, calcium, total protein, magnesium, phosphate, albumin, cholesterol, and uric acid. For renal function, an eGFR >90ml/min/m^2 will be considered normal using the CKD-EPI without albuminuria on dipstick.
    c. Liver function tests: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, conjugated bilirubin, gamma-glutamyl transferase [<1.5 x ULN (ALT, GGT, Total Bilirubin; local laboratory gender-specific reference ranges) will be considered not clinically significant].
    d. Negative HIV serology, Hepatitis B Surface Ag testing and hepatitis C serology.
    e. Females with a negative serum pregnancy test at screening and negative urine pregnancy test at Baseline and admission
17. Ex-smoker with a >10 pack year smoking history or a current smoker who is unable to stop smoking for the duration of the study.
18. History or presence of alcohol abuse (defined as regular alcohol consumption of more than 40g or 4 standard drinks per day) or drug habituation, or any prior intravenous usage of an illicit substance.
19. A positive urine drug test at screening or admission for confinement (e.g., amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, and opiates) unless there is an explanation acceptable to the Investigator (e.g. the participant has stated in advance that they consumed a

prescription or OTC product which contained the detected drug) and the participant has a negative urine drug screen on retest.

20. A positive alcohol breath test at screening or admission for confinement.
21. Known hypersensitivity or other contraindication to use of penicillin, azithromycin or any other beta-lactam or macrolide antibiotic(s).
22. Known hypersensitivity to soya protein.
23. Intolerance of throat swab procedure (exaggerated gag reflex).
24. Participation in a research study that involved blood sampling of more than 450 mL of blood, received or donated blood, blood products and/or plasma derivatives or any parenteral immunoglobulin preparation within 3 months of screening.
25. History of severe infections requiring hospitalisation for intravenous antibiotics (within the last 10 years). Exceptions to this would include a short course of intravenous antibiotics for appendicitis, biliary sepsis, diverticulitis and cellulitis.
26. History of cancer (except adequately treated squamous cell or basal cell carcinoma of the skin and cervical intraepithelial neoplasia).
27. Presence of implants (except for contraceptive implants) or prosthesis (e.g. artificial joints, pacemakers).
28. Receipt of another investigational product within the 30 days prior to screening involving an investigational product or other intervention that might affect risk of invasive S. pyogenes infection or compromise the integrity of the study (e.g. significant volumes of blood already taken in previous study).

29. Any significant disease or disorder which, in the opinion of the Investigator, may either put the participants at risk because of participation in the study, or may influence the results of the study, or the participant's ability to participate in the study.
30. Any employee of the sponsor or research site personnel directly affiliated with this study or their immediate family members defined as a spouse, parent, sibling, or child whether biological or legally adopted.

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page no.
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, name of intended registry	16
	2b	All items from the World Health Organization Trial Registration Data Set	16
Protocol version	3	Date and version identifier - *Not included in manuscript but text prepared based on HREC approved CHIPS trial protocol V1.0 Dated 16 April 2021*	
Funding	4	Sources and types of financial, material, and other support	18
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 18
	5b	Name and contact information for the trial sponsor	16
	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	18
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	14, Fig 3

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	6
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	9, Table 2
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, noninferiority, exploratory)	7
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 be obtained	7
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)	Supp
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10
	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)	12
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	12
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10
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	9, Table 2

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)	Fig 2
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	15
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	11
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	11
Methods: Data collection, management, and analysis			

Data collection methods	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	
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	12
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
Statistical methods	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	16
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	16
	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)	16
Methods: Monitoring			
Data monitoring	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 not needed	14, Fig 3
	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, Fig 3

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
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	14
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	16
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)	16
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable - *Not included in the manuscript but specified in the HREC approved study protocol*	
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	14
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
Access to data	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	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
	31b	Authorship eligibility guidelines and any intended use of professional writers - *Not included in the manuscript but specified in the HREC approved study protocol*	
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code - *Not included in the manuscript but specified in the HREC approved study protocol*	
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates - *Not included in the manuscript but specified in the HREC approved study protocol*	
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 - *Not included in the manuscript but specified in the HREC approved study protocol*	

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

BMJ Open

Study Protocol for Controlled human infection for penicillin G against Streptococcus pyogenes: a double blinded, placebo controlled, randomised trial to determine the minimum concentration required to prevent experimental pharyngitis (The CHIPS trial)

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Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Pharmacology and therapeutics
Keywords:	INFECTIOUS DISEASES, MICROBIOLOGY, CLINICAL PHARMACOLOGY

TITLE

Study protocol for Controlled human infection for penicillin G against *Streptococcus pyogenes*: a double blinded, placebo controlled, randomised trial to determine the minimum concentration required to prevent experimental pharyngitis (The CHIPS trial)

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Keywords: Group A Streptococcus, *Streptococcus pyogenes*, Human infection studies, Controlled human infection, Human challenge, Prevention study, Acute Rheumatic Fever, Rheumatic Heart Disease, Benzylpenicillin, Minimum Effective Dose

ABSTRACT:

Introduction:

Regular intramuscular benzathine penicillin G injections have been the cornerstone of rheumatic heart disease (RHD) secondary prophylaxis since the 1950s. As the pharmacological correlate of protection remains unknown, it is difficult to recommend changes to this established regimen. Determining the minimum effective penicillin exposure required to prevent *Streptococcus pyogenes* infection will accelerate development of new long-acting penicillins for RHD prevention as well as inform opportunities to improve existing regimens. The CHIPS trial will address this knowledge gap by directly testing protection afforded by different steady state plasma concentrations of penicillin in an established model of experimental human *S. pyogenes* pharyngitis.

Methods and analysis:

This is a double-blinded, placebo-controlled, randomised experimental human infection study. Sixty healthy adult volunteers aged 18 to 40 years will be recruited and randomised 1:1:1:1:1 to continuous intravenous penicillin infusions targeting five different steady state plasma concentrations of 0 (placebo), 3, 6, 12 and 20 ng/mL via a midline catheter. Each participant's penicillin pharmacokinetic parameters will be established prior to the challenge, to ensure accurate dosing for the continuous infusion. Following challenge with a well-characterised strain of *S. pyogenes*, participants will be observed for up to 6 days for development of pharyngitis and treated with antibiotics prior to discharge. The primary objective is to determine the minimum effective steady-state plasma penicillin concentration required to prevent experimental pharyngitis. Secondary objectives will explore systemic and mucosal immuno-inflammatory responses during pharyngitis, bacterial colonisation dynamics, environmental contamination, and qualitative evaluation of the participant experience.

Ethics and dissemination: Ethical approval has been obtained (Bellberry Human Research Ethics Committee). Findings will be reported in peer reviewed publications and presented at national/international stakeholder forums.

Trial Registration number: ACTRN12621000751875

STRENGTHS AND LIMITATIONS OF THIS STUDY:

1. The *S. pyogenes* controlled human infection model provides a unique platform for a randomised, double-blinded, placebo-controlled study to evaluate the minimum concentration of penicillin required to prevent infection.
2. The *S. pyogenes* challenge strain was selected after extensive characterisation efforts, including a reproducible penicillin minimum inhibitory concentration (12 ng/mL).
3. Individualised pharmacokinetic modelling will be used to determine the intravenous penicillin infusion dose required for each participant to achieve very low steady-state target plasma concentrations.
4. The sample size (n=60) is adequately powered to detect the anticipated effect size and far smaller than what would be required in any field trial designed to address these questions.
5. Despite the use of a standardised pharyngitis case definition to ascertain the primary outcome, individual assessor variability in assessment of severity and diagnosis of pharyngitis cannot be fully accounted for, reflecting the practical complexity encountered in clinical practice.

INTRODUCTION

Recurrent infections with *Streptococcus pyogenes* (Group A beta-haemolytic Streptococcus; *S. pyogenes*) are associated with development of acute rheumatic fever (ARF) and rheumatic heart disease (RHD).[1] RHD affects 40.5 million people globally and causes 306,000 deaths annually, mostly children and young adults living in low- and middle-income countries.[2, 3] The efficacy of intramuscular (IM) injections of 1.2 million units (MU; 900 mg) benzathine benzylpenicillin G (BPG) every three to four weeks for RHD secondary prophylaxis was first demonstrated in the 1950s and remains the only proven and cost-effective protection against recurrent infection and progressive RHD.[4-6] After deep IM injection, BPG is slowly hydrolysed to benzylpenicillin G (penicillin) and absorbed into the plasma.[7]

While secondary prophylaxis has been shown to be moderately effective in adherent individuals, poor adherence to painful monthly intramuscular injections (recommended for a minimum of 5 years) limits coverage of secondary prophylaxis and its overall effectiveness.[6, 8-10] There is an urgent need to improve penicillin formulations for patients with ARF and RHD. Stakeholder consultations with consumers and RHD experts have consistently identified the ideal characteristics for secondary prophylaxis which include reducing dose frequency (ideally every 3-6 months), reducing pain of administration, alternative delivery strategies (including injectable implants or non-injection methods) and cold chain independence as key aspirations for an acceptable product.[8, 11] However, as the pharmacological correlate of protection remains unknown, it is difficult to recommend changes to the established regimen.

It has conventionally been assumed that critical pharmacological correlate for prevention of *S. pyogenes* infections is the time between IM injections that plasma penicillin concentrations remain above 0.02 mg/L (20 ng/mL), a typical minimum inhibitory concentration (MIC) for *S. pyogenes* isolates.[12] However, emerging evidence from a number of high risk settings demonstrates that even the most adherent patients do not maintain these target concentrations for the majority of the interval between BPG

injections.[13, 14] Given the apparent efficacy in adherent patients, it is possible that current regimens of BPG confer protection at lower, sustained inter-injection levels of plasma penicillin. Alternatively, transient peaks in serum concentration may be sufficient as an intermittent presumptive treatment.

The opportunity to directly test the former hypothesis under the necessary controlled conditions has arisen with the advent of a new experimental human infection model of *S. pyogenes* pharyngitis in healthy adults.[15] The CHIPS trial will address a key knowledge gap by directly testing protection against experimental human pharyngitis, in relation to different steady state plasma concentrations of penicillin, to inform strategies for pharmacological secondary prophylaxis of RHD, including development of new and more effective long-acting penicillin formulations or optimising dose and dosing intervals with currently available formulations.

METHODS AND ANALYSIS:

Study Design

The CHIPS trial is a double-blinded, placebo-controlled, randomised human infection study to determine the minimum effective steady-state plasma penicillin concentration required to prevent pharyngitis following direct application of *S. pyogenes* to the oropharynx (Figure 1). Based on the successful human challenge model developed in the CHIVAS-M75 study,[15] healthy adult volunteers will be recruited through a private contract research organisation (CRO) and inoculated with the same *S. pyogenes emm*75 strain. A total of 60 participants will be recruited in 4 cohorts of 15 volunteers. Participants will be randomised 1:1:1:1:1 to receive continuous IV infusions of penicillin at 5 possible steady state plasma concentrations of 0 (placebo), 3, 6, 12 and 20 ng/mL. The study will be conducted within the CRO facility in Perth, Western Australia, with clinical support from a nearby tertiary hospital.

Patient and public involvement

The need to improve RHD secondary prophylaxis is underpinned by extensive consumer engagement which has consistently identified pain and frequency associated with BPG injections as barriers to adherence.[8, 16, 17] However, as this is a human infection study involving healthy volunteers, there is limited scope for health consumer input into the design and implementation of the study. While the methodology of our study involves *S. pyogenes* pharyngitis and its prevention using continuous penicillin infusion, the focus of our research and its intended beneficiaries are not sufferers of pharyngitis, but rather secondary prophylaxis for those living with ARF/RHD. Due to this indirect connection, involvement of target patient population would be premature. For the healthy volunteers who participate in the study, they will be made aware of results of this trial and informed of how to access the published findings.

Study objectives and outcomes

The primary objective is to determine the minimum plasma penicillin concentration associated with protection against experimental *S. pyogenes* pharyngitis following the challenge, assessed by the development of acute symptomatic pharyngitis (primary outcome) during the confinement period. This is assessed using the pharyngitis case definition from CHIVAS-M75 study, incorporating elements of clinical prediction rules based on Centor and McIsaac scores, change in tonsil size and real time molecular point-of-care test for *S. pyogenes* (ID NOW Strep A2, Abbott).[15] Secondary objectives are identification of plasma penicillin concentration required to prevent pharyngeal colonisation of *S. pyogenes*, and salivary penicillin concentration required to prevent *S. pyogenes* pharyngitis or colonisation. Exploratory objectives include characterisation of immune responses and inflammatory profiles comparing participants across penicillin dose bands and pharyngitis outcome, examination of *S. pyogenes* potential for environmental contamination (with relevance to disease transmission) and exploration of motivations and the lived experiences of the volunteers who take part in human infection studies (listed in Table 1 along with outcome/endpoint assessments).

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143 Table 1: Study objectives and outcomes

	Objective(s)	Outcome(s)/ Endpoint(s)
Primary	To determine the minimum plasma penicillin concentration required to prevent acute symptomatic *S. pyogenes* pharyngitis following a direct oropharyngeal challenge with *S. pyogenes* M75	Development of *S. pyogenes* pharyngitis during confinement period, according to a pre-defined clinical and laboratory criteria
Secondary	1. To identify the target plasma penicillin concentration required to prevent *S. pyogenes* colonisation of the pharynx	Development of *S. pyogenes* colonisation following challenge, defined as *S. pyogenes* M75 isolation from throat swab in absence of signs and symptoms of clinical pharyngitis after completing antibiotic treatment at conclusion of confinement period.
	2. To identify the target salivary penicillin concentration required to prevent *S. pyogenes* pharyngitis or colonisation	Assays to detect penicillin concentration in saliva from all participants
Exploratory	1. To characterise plasma humoral and cellular immunological profiles of immune response to experimental challenge with *S. pyogenes* in healthy participants	Laboratory assays to measure immunological and inflammatory responses to the challenge
	2. To characterise plasma inflammatory (CRP and procalcitonin) and metabolomic profiles of *S. pyogenes* pharyngitis	Measurement of inflammatory markers from blood samples
	3. To identify whether Cystatin C- based markers of renal function improve estimates of penicillin G renal clearance compared with creatinine-based measures	Measurement of Cystatin-C from blood samples
	4. To explore microbiological and local factors associated with *S. pyogenes* adhesion to tonsillar mucosa	Laboratory assays to measure mucosal response

	5. To explore *S. pyogenes* transcriptomic changes in response to penicillin exposure in *S. pyogenes* pharyngitis	Transcriptomic analyses/ genetic sequencing of *S. pyogenes* isolates
	6. To investigate potential environmental contamination of *S. pyogenes* via large respiratory droplets, airborne small respiratory droplets, and surface contact	Microbiological and culture analysis of participants' contact surfaces and surroundings
	7. To explore motivations, attitudes, and experiences of participating in clinical trials and human challenge studies	Reponses to questionnaires administered during study period by participants

Recruitment and eligibility

A database of healthy volunteers maintained by the CRO will be used for recruitment of study participants, along with multi-media advertisements (e.g., social media platforms of CRO and affiliated institutions) using materials approved by the ethics committee. Participants will be financially reimbursed of a value determined to be satisfactory by the ethics committee. Healthy adult males and non-pregnant, non-lactating females aged 18 – 40 years without pre-existing risk factors for severe *S. pyogenes* disease will be recruited. Strict eligibility criteria are in place to mitigate risks to potential participants (full eligibility criteria detailed in Supplementary Material 1; copy of the participant information sheet and consent form provided in Supplementary Material 2). In addition to usual 'healthy adult' inclusion and exclusion criteria, medical history and physical examination, prospective participants will undertake electrocardiography and transthoracic echocardiography to rule out undiagnosed sub-clinical cardiac pathology. They will also undergo screening throat swabs and a serum *emm*75 type-specific serology to exclude carriage and prior immunity to *S. pyogenes emm*75 strains, respectively.

Study Interventions

Dose-finding pharmacokinetic study

The overall journey of a participant from screening to completion of follow up is illustrated in Figure 2. At least several days prior to the inpatient challenge admission, each participant will have an individual pharmacokinetic dose-finding assessment. A 600 mg bolus dose of intravenous penicillin will be administered and serial venous blood samples will be collected for plasma penicillin concentration measurements at baseline, then 15, 30, 60, 120, 180, 240, and 360 minutes afterwards. Clearance and volume of distribution will be derived to enable calculation of individualised IV penicillin continuous infusion doses to attain the randomised target concentration for the *S. pyogenes* challenge admission.

Randomisation procedure

First 45 participants will be randomised 1:1:1:1:1 to one of 5 different target steady state concentrations (0 [placebo], 3, 6, 12 and 20 ng/mL). To ensure there is at least one participant in each concentration for each group of 5 and 3-per-concentration in each cohort of 15 participants, the volunteers will be block randomised in groups of 5 following an allocation sequence generated by the study statistician and stored on a secure server, accessible only to the unblinded pharmacy and analytical team members. All clinical staff and participants will remain blinded to the treatment allocation (concentration level) for the duration of the study.

Challenge procedures

Participants are considered enrolled from the time of commencing the penicillin infusion via a midline intravenous catheter on the day of admission (Day -1). On the day of challenge (Day +1), a sterile Dacron swab is dipped in a 1mL single-dose vial containing 1-3 x 10^5 colony-forming units (CFU) of *emm*75 *S. pyogenes* and applied directly to the participant's oropharynx. The single-dose vials will be produced according to the principles of Good Manufacturing Practice.[18, 19] Each participant will be challenged once

only, using a standardised procedure analogous to a diagnostic throat swab done 'in reverse', as previously described.[18] Participants will be fasted for 90 minutes before and after challenge.

Confinement and discharge

Following challenge, participants will remain inpatients at the CRO facility until reaching the primary pharyngitis outcome or until 5 days after challenge if they remain asymptomatic, whichever occurs first. The penicillin infusion will stop at that time and a separate oral antibiotic course will be initiated (azithromycin 500 mg once daily for 5 days[20]). All participants will be monitored as inpatients for at least 24 hours after their first dose of oral antibiotic prior to discharge. Subsequent safety follow-up visits will occur 7 and 28 days after the first dose of oral antibiotic. Participants will return unused antibiotic tablets which will allow monitoring of adherence to the remainder of oral treatment.

Adding/removing treatment arms

After 45 participants (3 cohorts), an interim analysis will be performed. Provided that the pre-specified statistical thresholds are met, the investigators may adjust the target concentration arms (while retaining the placebo arm) for the last cohort of 15 participants (to concentrations up to 100 ng/mL) to increase the precision of the minimum effective concentration estimate.

Pharmaceutical handling of penicillin

For each participant, individual pharmacokinetic parameters will inform the penicillin dose required to prepare the intravenous infusion bags for all possible dose allocations. After randomisation, infusion bags will be prepared at an aseptic compounding facility according to the individualised calculated dose to attain the allocated steady state plasma penicillin concentration. The stability of benzylpenicillin in 0.9% w/v sodium chloride IV infusion bags and the optimum sodium citrate concentration has been formally evaluated for the CHIPS trial (manuscript accepted[21]). These stability studies demonstrated excellent

chemical preservation of buffered benzylpenicillin at room temperature, with <1% degradation after 24 hours for benzylpenicillin 25 µg/mL in sodium citrate 100 µg/mL in 0.9% w/v sodium chloride solution, whether exposed to or protected from artificial light. Continuous infusion bags will be routinely changed every 24 hours. A sample of remnant fluid from each bag will be collected, stored at -80 °C, and assayed to confirm the expected stability of penicillin over the 24-hour period.

Measurement of possible environmental contamination and transmission potential

At 3 post-challenge timepoints (+24, +36 and +48 hours), Colistin Nalidixic Acid (CNA) agar plates will be placed in the participant's room for 4 hours to capture potential droplet or airborne transmission of *S. pyogenes*. Swabs will also be taken of the participant's surroundings and personal devices (approximately 25cm^2). To detect droplet transmission potential, participants will read a short text at each timepoint with CNA plates placed at varying lengths (30cm, 90cm and 180cm). All swabs and CNA plates collected will undergo transfer and processing for microbial culture for beta-haemolytic Streptococci (BHS) as per Clinical and Laboratory Standards Institute (CLSI) standards, including use of positive and negative controls. If no growth is detected after 24 hours, incubation will be extended for another 24 hours. Presence of *S. pyogenes* from beta-haemolytic colonies will be confirmed with agglutination kits using group specific antigens (Streptex™, Thermo Scientific).

Study participants' experience

Participants will complete surveys at 3 timepoints: admission, at diagnosis of pharyngitis or Day +3 (if asymptomatic), and immediately prior to discharge. These surveys will collect qualitative data using standardised questionnaires evaluating participant's motivation for involvement in the study and how expectations or concerns held prior to admission compared to the experience. In addition, participants will also be asked to keep a diary and record elements of their challenge admission specific to their

experience of participating in a human challenge study in a non-structured form. This qualitative data will be collected and collated into themes for analysis and reporting.

Governance

A unique governance structure, incorporating a Safety Data Review Team (SDRT), has been set up to meet the needs of this study as illustrated in Figure 3. Day-to-day conduct of the study and reporting of its progress to the SDRT is done by the trial management group whose members will remain blinded to the randomised allocation until after completion of study. SDRT has voting members (chaired by an independent expert) who make decisions regarding continuation of trial after each cohort of 15 is completed, with non-voting members from the analytical team and trial steering committee providing an advisory role. An independent study monitor will be engaged who will ensure that the investigation is conducted according to the protocol and regulatory requirements. Strict data management plan will be adhered to protect participant confidentiality in compliance with Good Clinical Practice guidelines.

Safety

As in the CHIVAS-M75[15] study, the following will be considered medically significant events in addition to the standard definitions – local and systemic complications of *S. pyogenes* infection, autoimmune sequelae of *S. pyogenes* infection (such as ARF, RHD, and glomerulonephritis), recurrent pharyngitis in participants caused by the challenge strain, and secondary cases of *S. pyogenes* infection with the challenge strain in non-participants.

Participant safety during confinement: Participants will be monitored closely during the confinement period in a purpose-built clinical trials facility with 24-hours clinical staffing and twice daily reviews. All adverse events will be recorded in real time and any serious adverse event (SAE) will be reported to the

SDRT within 24 hours of their occurrence. Starting a new cohort of participants will require approval by the SDRT following an interim review after each cohort's confinement period is completed.

Long term safety: The risk of long-term carriage of *S. pyogenes* is minimised with treatment using a non-beta-lactam antibiotic (azithromycin) prior to discharge. Additional reassurance comes from the CHIVAS-M75 study in which none of 25 participants challenged with the *emm*75 strain had developed persistent carriage, systemic or autoimmune complications of *S. pyogenes* at completion of 6 months follow up.[15] The risk of secondary spread of infection from participants will be negligible as they will be confined in a clinical trial facility with stringent infection control measures and will have had 24 hours of oral antibiotic treatment by the time of discharge back to community (in keeping with public health recommendations for school exclusion).

Challenge strain

The *S. pyogenes* challenge strain (*emm*75, M75) was isolated from a patient with pharyngitis in 2011. It has been extensively characterised and selected for its suitability for human challenge. It is an infrequent *emm*-type in most published series but reliably causes pharyngitis. The particular challenge strain has favourable antibiotic susceptibility, and does not have a virulence profile typical of hypervirulent strains.[19] The characterisation, selection, manufacture, storage, and quality assurance approach for the *emm*75 challenge strain *S. pyogenes* has previously been described.[15, 18] In the CHIVAS-M75 study, at the starting dose level of 1-3 x 10^5 CFU in each single-dose vial, the pharyngitis attack rate was 85%.[15]

Sample size calculation

Based on simulations, a maximum of 60 participants are required (recruited in 4 cohorts of equal size; starting with 5 treatment arms) to detect a minimum effective dose (MED) between 0-20 ng/mL, with >80% power and Type 1 error <5%. Trial simulations were based on: (i) an anticipated 25% of placebo participants symptom-free at the end of study Day +5; (ii) a monotonic normal dynamic linear model with

weakly informative prior distributions; (iii) equal allocation to all treatment arms; (iv) interim analyses after each cohort has completed (i.e. every 15 participants); (v) a high target of 90% symptom-free and a low target of 80% symptom-free in determining the MED; and (vi) stopping rules for success if the posterior probability that the MED is greater than the low target is greater than 80% (i.e. pr(MED>low target)>80%) and for futility if the posterior probability that the MED is greater than the upper target is less than 10% (i.e. pr(MED>upper target)<10%). Trial operating characteristics were calculated for 8 scenarios ranging from null efficacy to MED detected at the highest dose level.

Data analysis plan

Study data will be collected using paper and electronic source documents and managed using a secure institution hosted electronic database (Research Electronic Data Capture, USA). For the primary endpoint, Bayesian analyses will be performed on the accumulating data after each cohort completes study Day +5 and the primary pharyngitis endpoint is determined for each participant. It is anticipated that up to 25% of participants in the placebo arm may remain free from pharyngitis. A monotonic normal dynamic linear model will be used to assess the dose response and estimate the MED. After the completion of the second (n=30) and third (n=45) cohorts, we will evaluate stopping rules for success (pr(MED>low target)>80%) and for futility (pr(MED>upper target)<10%). All secondary outcomes will be summarised by treatment arm using appropriate statistics, including mean and standard deviation for continuous variables with symmetrical distributions or median and interquartile range for asymmetric distributions. Categorical variables will be summarised using frequencies and percentages.

ETHICS AND DISSEMINATION

This protocol (Universal Trial Number U1111-1264-9535) has been reviewed and approved by the Bellberry Human Research Ethics Committee (Ref: 2021-03-295) and is registered on the Australian New Zealand Clinical Trials Registry (www.anzctr.org.au, ACTRN12621000751875). The sponsor is the Telethon

Kids Institute and the study is indemnified under the existing institutional insurance policy. The results of the study will be of national and international significance. Our team has strong links to stakeholder groups and national and international profiles that will ensure dissemination of the results in peer-reviewed journals and presentation at relevant congresses.

DISCUSSION

The Global Resolution on Rheumatic Fever and Rheumatic Heart Disease calls for new technological approaches to improving global RHD control, including the "development of a long-acting formulation of penicillin that might improve secondary prophylactic regimens".[22] The CHIPS trial aims to address a key knowledge gap toward achieving this goal, by challenging the dogma regarding what is the MED of penicillin for successful prevention of *S. pyogenes* infection and secondary RHD prophylaxis.

Experimental human infection, or challenge studies, have been core platforms for infectious diseases research since at least Edward Jenner's 18th century smallpox vaccine studies.[23] In recent decades, reinforced by rigorous ethical frameworks and independent safety reviews, modern human challenge trials have contributed to accelerating development of new drugs, vaccines, and diagnostics.[23, 24] The CHIPS trial is the first to take advantage of the successful establishment of a human pharyngitis model in the CHIVAS-M75 study.[15] Although human challenge trials have previously tested the efficacy of drugs, the use of randomised steady-state plasma concentration levels of penicillin aiming to demonstrate a prophylactic threshold is an innovative approach among modern human challenge trials.[23-25] Even with a sample size of 60 participants, our study is adequately powered to detect the anticipated effect size.[26-29] The minimum effective dose of penicillin established in the present study is expected to provide evidence that sustained low plasma penicillin concentrations (<20 ng/mL) do prevent *S. pyogenes* infection while shedding further light on the relationship between serum and tissue penicillin concentrations and the role they might play in host-pathogen interaction leading to pharyngitis. Pharmacokinetic correlates of

protection identified will inform the target product profile for long-acting penicillin formulations and the development of implants or depot injections that provide a 'smoother' exposure profile to overcome the need for frequent painful injections associated with low adherence rates.[6, 8, 30]

Findings from the CHIPS trial may have wider applications beyond RHD secondary prophylaxis, for other indications for penicillin prophylaxis, such as recurrent lower extremity cellulitis. *S. pyogenes* is a leading cause of erysipelas and cellulitis, the latter commonly affecting older adults living in developed economies.[31-34] In the United States alone, it is estimated to cost $3.7 billion in healthcare expenditure from 14.5 million cases annually.[35] In addition, improved long-acting formulations of penicillin could have advantages for treatment of certain conditions including syphilis and other treponemal syndromes.

Potential limitations of our approach include the uncertain generalisability of a MED related to a single strain. Nonetheless, notwithstanding recent concerns stemming from rare penicillin binding protein mutations, *S. pyogenes* remains universally susceptible to penicillin.[36-38] The relationship between *in vitro* MIC of the *emm*75 challenge strain and the MED determined in the CHIPS trial will still be relevant to considering other strains with different MICs. Likewise, it is uncertain whether findings in healthy volunteers are generalisable to the relevant patient groups for RHD secondary prophylaxis. Certainly, future research will be needed to validate novel regimens of existing preparations or novel formulations which are underpinned by new knowledge obtained from this trial across a broad range of target patient populations. The CHIPS trial will also be a platform to build on insights into host-pathogen interactions already emerging from the CHIVAS-M75 trial, to exploring environmental determinants of transmission, and adding to the literature exploring the experience of participants in human challenge trials.[39, 40]

AUTHORS' CONTRIBUTIONS

Conceptualisation and design of the study: Laurens Manning, Jonathan Carapetis, Joshua Osowicki, Andrew Steer, Julie Marsh, Kevin Batty, Sam Salman, James McCarthy, Thomas Snelling, Joseph Kado, Thel Hla

Data analysis plan: Julie Marsh, Laurens Manning, Thel Hla

Drafting of the protocol: Thel Hla, Laurens Manning, Joshua Osowicki, Sam Salman, Julie Marsh, Renae Barr, Stephanie Enkel, Kevin Batty

Critical revisions of the manuscript: Thel Hla, Laurens Manning, Joshua Osowicki, Sam Salman, Kevin Batty, Julie Marsh, Joseph Kado, Renae Barr, Stephanie Enkel, Thomas Snelling, James McCarthy, Andrew Steer, Jonathan Carapetis

COMPETING INTERESTS STATEMENT

Investigators for this study have no financial or other competing interests.

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FIGURES

Figure 1: Pictorial synopsis of the CHIPS study. (Pen Css, penicillin steady state concentration; PK, pharmacokinetic)

Figure 2: Participants' journey through the CHIPS study.

Figure 3: Schematic illustration of governance structure and information flow. (AE – adverse event; CRO – contract research organisation; LCMS – liquid crystallography mass spectrometry; PI – principal investigator; PICC – peripherally inserted central catheter; SAE – serious adverse event)

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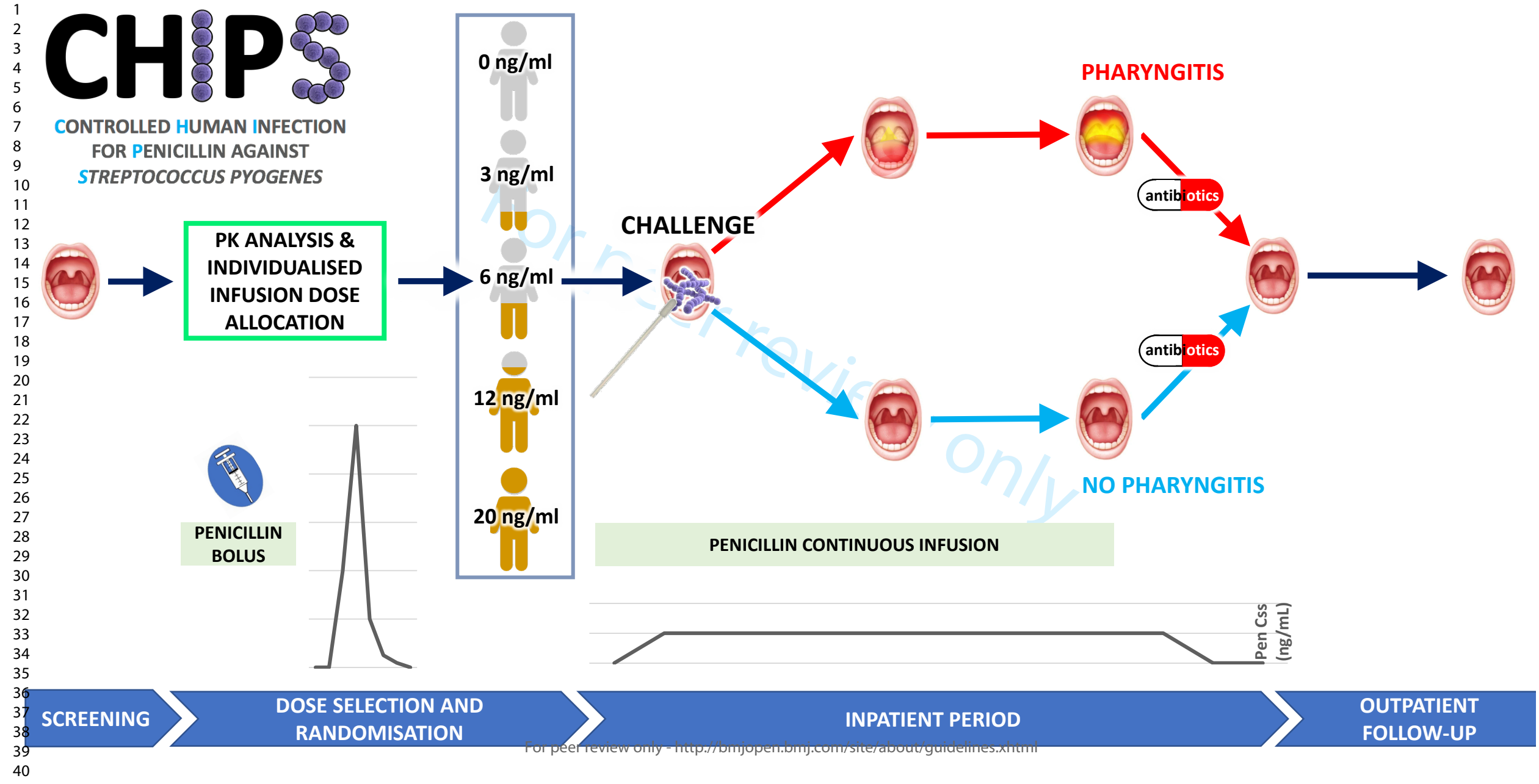

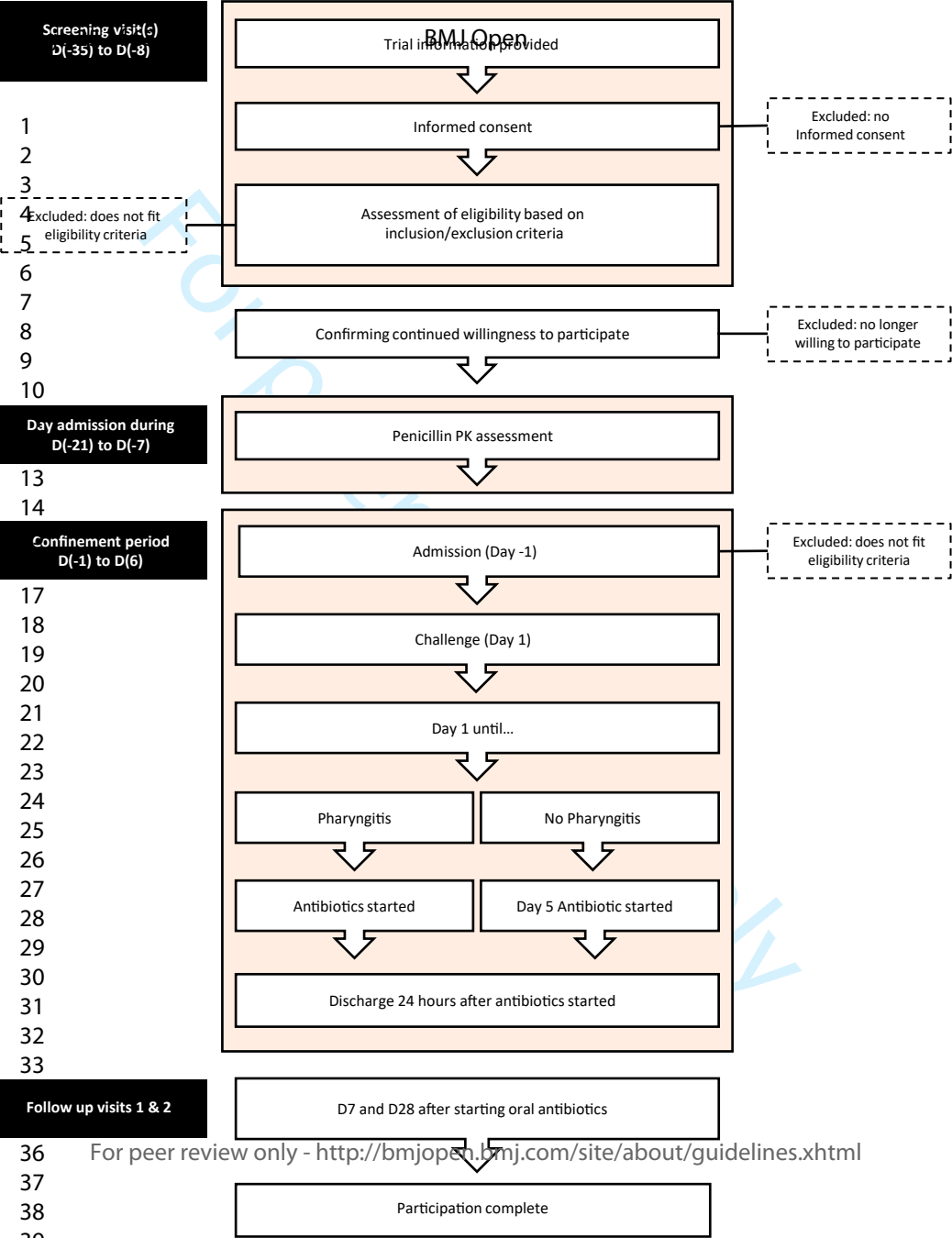

Trial Management Group

Day to day delivery & conduct of the trial:

- Not independent of trial
- Remains blinded to participant allocation, efficacy and safety reports for the entire trial

Comprising:

PI's delegate, PICC nurses, CRO Nursing/Medical Staff

Continue Trial: Yes/No

SAE/AE Reports

Primary outcome assessments

Individualised penicillin doses

Logistical issues

Safety Data Review Team (SDRT)

Voting members (mandatory attendance) comprising:

- **Independent medical monitor (Infectious Diseases Expert and SDRT Chair – preferably with experience in human challenge models)**
- **Independent *S. pyogenes* expert**
- **Independent trial statistician**
- **A/Prof Laurens Manning (PI)**

- Receives reports on SAE and AE with recommendations
- Provides recommendation to continue trial after each cohort of 15 participants
- Provides recommendation to modify treatment allocations with interim analyses with advice from non-voting team members

Non-voting members comprising:

Analytical Team (mandatory attendance):

Pharmacometrician, trial biostatistician, LCMS analyst

- Not-independent of trial
- Responsible for treatment allocation, individualised dosing & planned interim analyses
- Provides advice to voting members

Trial steering committee (invitees, as required attendance):

Listed CHIPS Investigators

- Not independent of trial
- Executive advice to voting members which considers the interests of trial, participants, funder, sponsor

SUPPLEMENTARY MATERIALS

Inclusion and exclusion criteria for eligibility to participate in CHIPS study

Inclusion criteria
1. Males or females, aged 18 - 40 years (inclusive).
2. Body mass index of 18.0 - 32.0 kg/m^2, inclusive, and body weight ≥ 50.0kg
3. Medically healthy, determined by medical history, physical examination, transthoracic echocardiogram, laboratory profiles without any clinically significant abnormalities, vital signs, and 12-lead ECG at screening.
4. Systolic blood pressure (SBP) of 90 mmHg – 140 mmHg and diastolic blood pressure (DBP) of 40 mmHg - 90 mmHg.
5. Resting heart rate (HR) of 40-100 bpm
6. Females must be non-pregnant, non-lactating or postmenopausal.
7. Females of childbearing potential must agree to use a barrier method for the duration of study
8. Must be willing and able to read, understand, and sign the participant information and consent form. Willing to comply with all study requirements.
9. Willing to abstain from the use of mouthwash from the day of screening until the first outpatient visit.
10. Must be willing for insertion and have adequate sites for placement of indwelling intravenous cannulae and midline intravenous catheter.
Exclusion criteria

1. Evidence of pre-existing immunity to the challenge strain, defined for this study as a high serum IgG to a peptide comprising the first fifty amino acids of the M75 protein (N-terminal hypervariable region) measured by ELISA.
2. Currently taking penicillins or use of any penicillin-based antibiotics from screening through to the final study visit. The use of probenecid, NSAIDs, or other medications which may significantly alter penicillin PK will also not be permitted within 14 days prior to study drug administration until completion of the final follow-up visit.
3. Any corticosteroid, immunomodulator or anticoagulant use in the previous 3 months, antiplatelet use in the previous 2 weeks, or anticipated use of such drugs during the study period. Any participant currently receiving or having previously received immunosuppressive therapy, including systemic steroids including adrenocorticotrophic hormone (ACTH) or inhaled steroids in dosages which are associated with hypothalamic-pituitary-adrenal axis suppression such as 1 mg/kg/day of prednisone (or its equivalent) or chronic use of inhaled high potency corticosteroids (budesonide 800 µg or fluticasone 750 µg per day). Intranasal corticosteroid use is not allowed from 14 days prior to admission, during the confinement period, and is discouraged prior to the first outpatient visit. Topical corticosteroid use is allowed.
4. Use of prescription or non-prescription drugs (except for oral contraceptive pill in healthy adult females) and herbal supplements (such as St John´s Wort) within 14 days or 5 half-lives (whichever is the longer) prior to the inoculation administration.
5. History of any clinically important cardiac, endocrine, haematological, hepatic, immunologic, metabolic, urologic, pulmonary, neurologic, dermatologic, psychiatric, and renal, or other major disease.
6. History of hospitalisation for illness within the six months prior to enrolment into study, or major surgery within the 12 months prior to enrolment into study.

7. History of tonsillectomy, adenoidectomy or splenectomy.
8. Known or suspected autoimmune disease or impairment/alteration of immune function resulting from:
    a. Congenital or acquired immunodeficiency (including IgA deficiency)
    b. Receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within the preceding 6 months.
9. History of a severe drug reaction or severe allergic reaction (eg. anaphylaxis, convulsions) or clinically significant allergic disease diagnosed by a Physician.
10. Personal or family history of severe *S. pyogenes* infection or sequelae (such as acute rheumatic fever, rheumatic heart disease, post-streptococcal glomerulonephritis) or invasive *S. pyogenes* disease (toxic shock syndrome, necrotizing fasciitis, bloodstream infection, pleural empyema, meningitis, puerperal sepsis).
11. Clinically significant disease or any condition or disease that might affect drug absorption, distribution, or excretion, e.g. gastrectomy, diarrhoea.
12. Any vaccination within the last 28 days (except for COVID-19 or seasonal influenza) or use of any antibiotic therapy during the 14 days before challenge.
13. Presence of an acute infectious disease or febrile illness (e.g., sub-lingual temperature ≥ 37.5°C) within the five days prior to challenge with *S. pyogenes* M75.
14. Significant acute or chronic infection within 14 days prior to inoculation that the Investigator deems may compromise participant safety.
15. Any clinically significant abnormal finding on biochemistry or haematology blood tests, urine analysis, ECG or transthoracic echocardiogram at screening.
16. Laboratory tests that fail to meet the following thresholds:

a. Haematology: Haemoglobin, haematocrit, red cell count, white cell count with differentials, platelet count, MCH, MCV, MCHC– parameters within gender-specific reference intervals from the local laboratory unless deemed not clinically significant by the investigator.

b. Clinical chemistry within gender-specific reference intervals from the local laboratory unless deemed not clinically significant by the investigator: urea, glucose, creatinine, sodium, potassium, chloride and bicarbonate, lactate dehydrogenase, calcium, total protein, magnesium, phosphate, albumin, cholesterol, and uric acid. For renal function, an eGFR >90ml/min/m^2 will be considered normal using the CKD-EPI without albuminuria on dipstick.

c. Liver function tests: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total bilirubin, conjugated bilirubin, gamma-glutamyl transferase [<1.5 x ULN (ALT, GGT, Total Bilirubin; local laboratory gender-specific reference ranges) will be considered not clinically significant].

d. Negative HIV serology, Hepatitis B Surface Ag testing and hepatitis C serology.

e. Females with a negative serum pregnancy test at screening and negative urine pregnancy test at Baseline and admission

17. Ex-smoker with a >10 pack year smoking history or a current smoker who is unable to stop smoking for the duration of the study.

18. History or presence of alcohol abuse (defined as regular alcohol consumption of more than 40g or 4 standard drinks per day) or drug habituation, or any prior intravenous usage of an illicit substance.

19. A positive urine drug test at screening or admission for confinement (e.g., amphetamines, barbiturates, benzodiazepines, cannabinoids, cocaine, and opiates) unless there is an explanation acceptable to the Investigator (e.g. the participant has stated in advance that they consumed a

prescription or OTC product which contained the detected drug) and the participant has a negative urine drug screen on retest.

20. A positive alcohol breath test at screening or admission for confinement.
21. Known hypersensitivity or other contraindication to use of penicillin, azithromycin or any other beta-lactam or macrolide antibiotic(s).
22. Known hypersensitivity to soya protein.
23. Intolerance of throat swab procedure (exaggerated gag reflex).
24. Participation in a research study that involved blood sampling of more than 450 mL of blood, received or donated blood, blood products and/or plasma derivatives or any parenteral immunoglobulin preparation within 3 months of screening.
25. History of severe infections requiring hospitalisation for intravenous antibiotics (within the last 10 years). Exceptions to this would include a short course of intravenous antibiotics for appendicitis, biliary sepsis, diverticulitis and cellulitis.
26. History of cancer (except adequately treated squamous cell or basal cell carcinoma of the skin and cervical intraepithelial neoplasia).
27. Presence of implants (except for contraceptive implants) or prosthesis (e.g. artificial joints, pacemakers).
28. Receipt of another investigational product within the 30 days prior to screening involving an investigational product or other intervention that might affect risk of invasive *S. pyogenes* infection or compromise the integrity of the study (e.g. significant volumes of blood already taken in previous study).

29. Any significant disease or disorder which, in the opinion of the Investigator, may either put the participants at risk because of participation in the study, or may influence the results of the study, or the participant's ability to participate in the study.
30. Any employee of the sponsor or research site personnel directly affiliated with this study or their immediate family members defined as a spouse, parent, sibling, or child whether biological or legally adopted.

PARTICIPANT INFORMATION SHEET & INFORMED CONSENT FORM

Study Name:	Controlled human infection for penicillin against *Streptococcus pyogenes*: a double blinded, placebo controlled, randomised trial
Protocol No:	1111-1264-9535
Study Sponsor:	Telethon Kids Institute
Study Doctor:	Associate Professor Laurens Manning
Study Site:	Linear Clinical Research

You are being invited to take part in a research study. This is because you are 18 to 40 years of age and in good general health. This Participant Information Sheet and Informed Consent Form (PICF) has information to help you decide if you want to participate.

Please take your time to read this document carefully and ask the study doctor or staff any questions you may have, and to explain any words, terms, or sections that are unclear to you. You should not sign this form until you understand all of the information presented in the following pages and until all of your questions about the research have been answered to your satisfaction.

You will be asked to complete a simple multiple-choice quiz prior to signing the consent form to confirm that you understand all of the information presented to you, if you select an incorrect answer a staff member will explain which is the correct answer and why the answer you chose was incorrect, incorrect answers will not prevent you from joining the study.

Your participation in this study is entirely voluntary and you may withdraw at any time. If you decide to participate in this study, you can then choose to stop taking part in the study at any time for any reason. You are encouraged to discuss the study with your family and friends prior to signing consent. We also recommend discussing your participation with your General Practitioner (GP), should you have one, especially if you are taking any prescription medication.

Linear Clinical Research Ltd will be paid by the Sponsor, Telethon Kids Institute, for conducting this study.

Study Synopsis

We are conducting this study to find out the minimum level of penicillin (an antibiotic) in your blood required to prevent pharyngitis (a sore throat caused by bacteria) due to infection by *Streptococcus pyogenes* (Strep A - a type of bacteria). To achieve this, we will be giving participants different doses of benzylpenicillin (a type of penicillin antibiotic) administered

intravenously (directly into a vein) and then exposing them to the Strep A bacteria to see whether or not the participant gets a sore throat. Although a sore throat is something that is normally easily recovered through treatment by antibiotics, previously untreated Strep A infections can leave patients exposed to further unwanted side effects or more severe diseases if they are re-infected (described below). When this occurs (typically in vulnerable children in areas of socioeconomic disadvantage), patients usually require deep, painful, monthly injections of penicillin for a minimum of 10 years. We would like to identify more effective treatment strategies for these patients, which involve less pain and less frequent interventions. To come up with this solution, it is crucial for us to know what level of penicillin is required to prevent re-infection. This will allow us to calculate the total amount of penicillin required and also the correct release rate to be used in alternate treatment strategies.

Why is this study important and what is involved?

Streptococcus pyogenes or Strep A is a bacterium that commonly causes a sore throat and skin sores in susceptible people. It is usually a mild illness cured with a course of appropriate oral antibiotics. However, when Strep A infections are not properly treated with antibiotics (i.e. in areas of socioeconomic disadvantage), the body's own response to Strep A can lead to a condition known as acute rheumatic fever (ARF). ARF is rare in industrialised settings with access to a high standard of living and sanitation. However, people living in socioeconomic disadvantage with poor access to healthcare (such as children in low- and middle-income countries or Indigenous Australians) remain vulnerable to it. ARF typically develops 2-4 weeks after Strep A infection and symptoms include joint swelling and pain, fever, uncontrolled movements, and skin rash. Untreated and over time, repeated episodes of ARF can lead to damage of the heart valves, also known as rheumatic heart disease (RHD). RHD affects an estimated 33.4 million people worldwide, resulting in the death of approximately 319,400 people each year. In Northern Australia, Indigenous populations are particularly affected due to the socioeconomic disadvantages prevalent in these communities such as inadequate access to healthcare and overcrowding due to inadequate housing.

Benzylpenicillin is marketed in Australia as BENPEN™ and is a penicillin antibiotic that has been approved by the Therapeutic Goods Administration (TGA) for the treatment of other infections such as streptococcal infections, infections of the upper respiratory tract, syphilis, meningitis and some skin diseases.

BENPEN™ is an antibiotic that belongs to a group of medicines called penicillins. Another longer-acting form of penicillin (called Benzathine Penicillin G) is typically given monthly by deep, painful injection into the thigh muscles of patients who have previously been diagnosed with ARF or RHD to prevent further Strep A infection. However, many patients who need this treatment (often young children and teenagers) will fail to attend these injections because of the associated pain and frequency. If we can develop more effective and tolerable treatments (i.e. longer acting and less painful), this may increase patients' willingness to receive this vital

treatment. Therefore, we are investigating the minimum blood penicillin level that offers effective protection against Strep A re-infection by using sore throat caused by Strep A to represent the more serious conditions such as acute rheumatic fever and rheumatic heart disease.

Through this research study, we hope to find out the minimum penicillin concentration in a person's bloodstream that will prevent Strep A pharyngitis. We want to find out what effects it has on you and your health as healthy adult males and females.

The design of this study is a double-blind, randomised and placebo-controlled challenge study.

A double-blind study means that the study doctor and the participants who take part won't know which group is getting Benzylpenicillin and which group is getting placebo. This way, the findings from the two groups will be treated equally. Neither you nor the study doctor will know which group you are in. However, this information can be obtained in case of an emergency.

To be randomised means that a computer will put participants into groups by chance. You are 4 times more likely to receive study drug than placebo. If the number of participants in the total group decreases, then this could mean that your chances of receiving the active study drug are higher. Neither you nor the study doctor can choose what you receive.

This study will compare Benzylpenicillin with placebo. The placebo will be similar to the penicillin treatment, but with no active drug in it. One group of participants will take Benzylpenicillin and another group will take the placebo. The effects of the study drug will be compared to participants who are taking the placebo.

A challenge study means you will be deliberately exposed to an infectious agent to see if the dosage of study drug you receive is capable of preventing symptomatic infection. In this study you will receive a challenge of Strep A bacteria applied to your throat to evaluate whether the dosage of Benzylpenicillin that is received is effective at preventing symptomatic infection.

We will be testing a continuous infusion of the study drug in up to 60 healthy volunteers. The first 45 of the participants will be divided into 5 groups of 9 people each. The dose level received by the final 15 participants will be determined based on the results of the initial groups. The following is the way that the groups are planned to be dosed:

Group	Number of Participants	Dose level (ng/mL)
A	9	20
B	9	12
C	9	6
D	9	3
E	9	0 (placebo)
F	15	TBC

The dose level may vary based on the safety information that is reviewed by the safety monitoring committee, but you would be informed if this was the case. You will be informed of the actual dose that you will receive, bearing in mind that you may receive placebo.

The study drug will be administered via a continuous infusion for at least 24 hours into your arm. After at least 12 hours of infusion you will be administered the Strep A challenge via oropharyngeal (to the top of your throat) swab. You will not be required to fast prior to your continuous infusion but you will be required to fast from food and water for 90 minutes prior to and following the Strep A challenge.

This study is being conducted by Linear Clinical Research Ltd. The study is being sponsored in Australia by Telethon Kids Institute.

Your GP will be notified of your participation in this study and of any clinically relevant information.

What will I be asked to do?

If you choose to take part in this study and it is determined you are eligible and able to participate, your length of involvement would be up to approximately 61 days.

You would be required to attend a Screening Visit up to 35 days before your scheduled challenge. You will also be required to attend the clinic for the day for a baseline visit up to 21 days prior to the scheduled challenge to determine how quickly your body gets rid of the penicillin to evaluate how much you will receive on dosing day. You will check-in the day before your scheduled challenge to begin your continuous infusion of the study drug and may remain in the clinic for a maximum of 6 days and 6 nights. Your health will be evaluated prior to discharge from the clinic, you may be discharged earlier than expected if you develop pharyngitis. Follow-up visits will be performed 7 and 28 days after starting the oral antibiotics. During your stay at the clinic, all meals and refreshments will be provided for you, so please inform the staff if you have any special dietary requirements.

Additional volunteers will be recruited and admitted to the unit as alternates in order to ensure we are able to dose the required number of participants.

Please note that if you are deemed eligible following the screening and baseline visits, this will not automatically mean you will be included in the study, with additional eligibility checks needing to occur on the day of baseline and Day -1. If you have been admitted to the unit for the baseline visit and are not required for the confinement period, you will be considered an alternate. You will be advised whether or not you will be required by the study team prior to check-in on Day -1.As well as alternates, there may be some participants who are admitted to the unit and are not required for dosing. If you are not required for dosing on Day 1 you will receive a partial payment of $400.

What will happen during the study visits?

Screening Visit – (Day -35 to Day -8; visit should be about 3-4 hours)

This visit will occur up to 34 days before your entry into the study and will involve:

- A discussion with the study doctor to make certain you fully understand the study, its procedures and requirements. Please make sure you ask any questions you may have about the study before or during this visit. After completing a simple quiz to confirm that you understand what will occur during the study, you will need to sign the attached Informed Consent Form to confirm you are willing to participate in this study and follow all instructions provided by the study staff, as well as abide by any study restrictions (these are detailed in the 'What are my responsibilities in this study' section).

  Please note that you will have the opportunity to receive this participant information sheet and consent form prior to coming into the clinic for the screening visit. This will allow you to review the information and discuss your potential involvement in this study with family, friends and/or a medical professional of your choosing (such as your GP). After signing the consent form in the presence of the study doctor you will have the opportunity to leave the clinic and come back at a later time, to complete the screening visit assessments outlined below, should you wish to discuss your involvement further with friends, family or a medical professional of your choosing.

- Following your consent, you will undergo a complete medical examination which will include:
  - Documentation of demography (race, gender, ethnicity);
  - Description of your medical and surgical history. Please ensure you inform the study doctor of any important family history and describe your personal history in full including any periods of hospitalisation, illness, surgery, blood

donations and drug reactions/allergies as these may affect your capacity to safely participate in this study;

- A full physical examination. Your overall health will be assessed which may include assessment of your general appearance, head, neck, ears, eyes, nose, throat, skin, cardiovascular system, respiratory system, gastrointestinal system, and neurological system; height and weight will also be measured. You will not be required to undress for this examination but may be required to move or lift parts of your clothing out of the way to allow examination of the chest, back and abdomen. For your privacy, this will be done behind a closed curtain;
- Electrocardiogram (ECG; a recording of the electrical activity of the heart), which involves placement of painless sticky pads (or electrodes) onto your chest, arms and legs to assess the electrical activity of your heart. You will be required to remove or lift your clothing out of the way to allow placement of the electrodes on your chest area and this will be done behind a closed curtain for your privacy;
- Vital sign measurements (blood pressure, pulse rate, breathing rate and temperature).

- You will be asked about any medications that you have taken in the last 6 months, or any other medications or products that you are currently using (including alcohol and tobacco use), as some medications must not be taken before or during the study (please refer to the 'What are my responsibilities in this study' section for medications to avoid during the study).
- You will be required to give a urine sample that will be used to perform tests to assess your general health, including screening for drugs of abuse. Please note you will not be eligible to participate in this study if you return a positive test result.
- Breathalyser samples for alcohol use will be obtained, please note you will not be allowed to participate in the study if you return a positive result.
- You will be asked how you are feeling. Please make sure you tell study staff as much information as possible.
- A sample of blood (approximately 12 mL, or 3 teaspoons) will be taken from a vein in your arm with a needle and syringe – this will be used to perform tests to assess your general health. If you are female, one of these samples may also be used to perform a pregnancy test or a follicle stimulating hormone (FSH) test to confirm post-menopausal status. Please note you will not be eligible to participate in this study if you return a positive pregnancy result.
- In order to take part in the study, you must agree to have specific serology testing for Hepatitis B, Hepatitis C and Human Immunodeficiency Virus (HIV). Approximately 5 mL, or 1 teaspoon of blood, will be taken for this testing (included in the volume above). You will receive information and counselling before the test. If a positive result

is found as a result of this testing, appropriate counselling services will be arranged by the study doctor to discuss treatment options. You will not be eligible to participate if you return a positive test result. Please note that as per Western Australian Law, Hepatitis B, Hepatitis C and HIV are notifiable diseases, and a positive result will be notified to the Department of Health.

- An additional sample of blood (approximately 4mL, or one teaspoon) will be taken to assess for any pre-existing immunity to Strep A. You will not be eligible to participate if you return a positive test result.
- You will be tested for bacterial infection via 3 throat swabs. To collect the swab sample, you will be asked to tilt your head back prior to the swab being inserted into your mouth towards the back of your throat. The swab will be rotated several times and then removed.
- At the conclusion of screening, provided you are eligible and willing to participate in the study, a study staff member will contact you and explain the details of the study to you, including re-confirming the study dates and restrictions.
- After your screening visit, you will be required to undergo an echocardiogram. An echocardiogram uses ultrasound waves to produce images of your heart. This commonly used test allows the measurement of the size of different parts of your heart and to see how your heart is beating and pumping blood. The study team will assist in booking your echocardiogram for you and provide you with directions on how to get there.
- There may be reasons why you are not allowed to take part in this study. The study doctor or staff will discuss these with you.

Individualised Penicillin Assessment – (Day -21 to Day -8; visit should be about 7-8 hours)

This baseline visit will occur up to 20 days before your entry into the study and will involve a number of assessments to determine how quickly your body gets rid of penicillin. This will allow the study team to determine the exact dose of penicillin required on Day -1.

The following assessments will be performed at various times throughout the day:

- Physical examination
- Measurement of your height and weight
- Measurement of your vital signs
- Blood samples (up to 8 mL or approximately one and a half teaspoons each time) will be taken for tests to assess your general health.
- Additional blood samples (up to approximately 15 mL or three teaspoon each time) will be taken for research purposes. Samples obtained for research purposes will give us better insight into how your body reacts to Strep A infection, how your body gets rid of penicillin and how Strep A reacts to penicillin.
- Additional blood samples (up to approximately 5 mL or one teaspoon each time) will be taken to measure levels of penicillin in your blood (PK sample).

- If you are a female a sample of urine may be collected to perform a pregnancy test (please note you will not be allowed to participate in the study if you return a positive pregnancy result)
- You will be regularly asked how you are feeling and if you had any changes in your health or taken any new medications. It is very important to tell the study staff about anything that has changed so that it can be properly recorded before you have any study medication. These changes won't necessarily keep you from continuing in the study, so please make sure you tell study staff as much information as possible.
- An indwelling cannula (a flexible, small plastic tube) will be inserted into a vein in your arm to allow for the collection of blood before the penicillin is administered and at specific intervals up to 6 hours after administration. If the cannula becomes blocked or stops working, some of the blood samples which were planned to be collected in this way may be collected with a needle and syringe.
- A second indwelling cannula will be inserted into a vein in your arm for the delivery of the penicillin. Penicillin will be administered via infusion over 2 minutes. The study doctors will use the data collected from this procedure to decide on the amount of penicillin to administer on Day -1.
- You will be provided with meals during your inpatient stay.

Treatment Period (Day -1 up to Day 6)

You will check in to the Linear study clinic the day that you will receive the continuous penicillin infusion (i.e. Day -1) and will be assessed to confirm your ongoing eligibility to participate in the study. Please note that in order to protect members of the public from infection you will be confined to a separate ward to our other participants. You will be permitted to leave this ward in emergencies and when you need to use the bathroom. The study team will explain this to you in more detail.

The following assessments will be performed at various times during your stay at the Linear study clinic:

- Physical examination
- Measurement of your vital signs
- Blood samples (up to approximately 8 mL or one and a half teaspoons each time) will be taken for tests to assess your general health.
- Additional blood samples (up to approximately 3 mL or slightly less than one teaspoon each time) will be taken for research purposes.
- Additional blood samples (up to approximately 5 mL or one teaspoons each time) will be taken to measure levels of penicillin in your blood (PK sample).
- A urine sample will be collected to test for drugs-of-abuse. If you are a female this sample may also be used to check for pregnancy (please note you will not be allowed to participate in the study if you return a positive pregnancy or drugs-of-abuse result).

- Breathalyser samples for alcohol use will be obtained at check-in, please note you will not be allowed to participate in the study if you return a positive result.
- You will be tested for bacterial infection via throat swabs. To collect the swab sample, you will be asked to tilt your head back prior to the swab being inserted into your mouth towards the back of your throat. The swab will be rotated several times and then removed.
- Saliva samples will be collected to measure penicillin levels. You will be asked to rinse your mouth with water and then fast for 60 minutes prior to placing a cotton swab in your mouth. You will then be asked to move the cotton swab around in your mouth until it is saturated.
- Nasal lining fluid will be collected via special collection device. An absorbent strip of the device will be inserted into your nostril for approximately 60 seconds.
- Photographs of the back of your throat will be taken. You will be asked to stick out your tongue and say 'ah' in order to capture the best view of your throat for the photo. If required, the investigator will use a wooden tongue depressor to gently push down on your tongue for a better view.
- You will be given a questionnaire to complete at 3 timepoints (Day -1, Day 3 and Day of Discharge) during your confinement, it is estimated that each timepoint will take up to 30 minutes to complete, you are encouraged to complete these questionnaires in your own time. You will also be given a diary to log your feelings to complete during your stay. If you have any questions about the questionnaires or diary you can contact a CHIPS study representative on (08) 6319 1456 or (08) 6319 1254 for assistance.
- On Day -1 a midline intravenous catheter will be inserted into the middle upper arm under guidance via ultrasound. A midline catheter is an 8-12cm catheter that sits in your vein for drug delivery over multiple days. You will be administered an individualized loading dose of penicillin initially for 2 minutes followed by continuous administration for a maximum of 5 days. The study team will inspect the catheter every 12 hours following insertion.
- On Day 1 after at least 12 hours of continuous penicillin administration you will be 'challenged' with Strep A bacteria. This will involve your throat being swabbed to apply the bacteria. You will be required to fast from food and water for 90 minutes prior to and after the challenge. Following challenge you will be assessed by the study doctor every 12 hours to monitor for pharyngitis, if you have a sore throat at all during the study you will be offered pain relief medication (paracetamol) to ease your symptoms.
- If you develop pharyngitis from the Strep A challenge the continuous penicillin will be stopped and you will be administered non-penicillin oral antibiotics (azithromycin). If you do not develop pharyngitis, on Day 5, the continuous penicillin will be stopped and you will be administered non-penicillin oral antibiotics. During your stay you will be able to request paracetamol at any given time to control any symptoms you may be feeling.

- You will be regularly asked how you are feeling and if you had any changes in your health or taken any new medications. It is very important to tell the study staff about anything that has changed so that it can be properly recorded before you have any study medication. These changes won't necessarily keep you from continuing in the study, so please make sure you tell study staff as much information as possible.
- You will be provided with meals during your inpatient stay. You will be required to avoid eating for at least 90 minutes prior to challenge and for 90 minutes after the challenge.
- 24 hours after starting your oral antibiotics (azithromycin), if the study doctor approves, you will be checked out of the clinic and can return home. Please make sure you let the study doctor or study staff know of any symptoms you may be feeling before leaving the clinic, so the study doctor can properly assess you before you leave.

Follow-up Visits (7 and 28 days post oral antibiotics) (Visit should be approx. 2 hours)

You will need to return to the study clinic for a follow-up visit 7 and 28 days after starting your 5 day course of oral antibiotics and may undergo the following procedures:

- Physical examination
- Measurement of your vital signs
- Blood samples (up to approximately 8 mL or one and a half teaspoons each time) will be taken for tests to assess your general health.
- An ECG will be performed
- You will be required to give a urine sample that will be used to perform tests to assess your general health.
- Additional blood samples (up to approximately 3 mL or 0.5 teaspoons each time) will be taken for research purposes.
- Saliva samples will be collected. You will be asked to rinse your mouth with water and then fast for 60 minutes prior to placing a cotton swab in your mouth. You will then be asked to move the cotton swab around in your mouth until it is saturated.
- You will be asked how you are feeling and if you have had any changes in your health since dosing and whether you have taken any other medications.

	Screening	Individualized Penicillin Assessment (Baseline)								Confinement										Outpatient Follow Up	
Day	D(-35) to -8									-1	1				2	3	4	5	6	7 Days post A/B	28 Days post A/B
Hours		0	0.25	0.5	1	2	3	4	6		0	2	12	24							
Review eligibility	x	x								x											
Informed consent	x																				
Survey questions & Diary										▼											
Medical history	x	x																			
BMI	x	x								x											
Vital signs	x	x						x		x	x[1]				x[1]	x[1]	x[1]	x[1]	x[1]	x	x
Physical Examination	x	x								x	x				x	x	x	x	x	x	x
Echocardiogram	x																				
Breathalyser & Urine drug screen	x									x											
Urinalysis	x																				x
ECG	x																				x
Clinical Lab tests	x	x								x											x
Pregnancy test	x	x								x											
Midline insertion										x											
Penicillin Administration		x								x	x[2]										
PK Sample		x	x	x	x	x	x	x	x	x	x		x	x	x	x[2,3]	x[2,3]	x[2,3]			
Research Bloods		x								x		x	x	x	x	x	x	x		x	x
Strep A challenge											x										
Throat swabs	x									x				x	x	x[3,4,7]	x[3,4,7]	x[3,4,7]	x[3,4,7]	x	x
Photo of throat										x						x[5]					
Nasal lining fluid										x		x	x	x	x						
Saliva Sample										x						x[6]				x	x
Oral antibiotic (A/B)																x[5]				If required	
Adverse Events and Symptoms		▼																		x	x
Current Medications	x	▼																		x	x

Study Schedule of Assessments

1: 4 Times a day | **2:** Until diagnosis or Day 5 | **3:** 12 hourly | **4:** Until Discharge | **5:** At diagnosis or Day 5 | **6:** At Start of Oral A/B and at discharge | **7:** At sore throat

What are my responsibilities in this study?

The following things are important during your participation in this study:

- Please inform the study doctor of any medications, including herbal, vitamin, over the counter or prescription you have taken in the month prior to the expected dosing day, as these will need to be recorded and will help to determine your eligibility for study participation.
- You will not be permitted to take any non-study penicillin-based antibiotics from screening through to the end of the study. You will also not be permitted to take probenecid or Non-Steroidal Anti-Inflammatory Drugs (NSAIDs - e.g. ibuprofen) within 14 days prior to penicillin administration. Sporadic ibuprofen use (less than 5 occasions) over the 14 days is permissible.
- You must not have been vaccinated 28 days or used any antibiotic therapy 14 days prior to your challenge, except for seasonal flu or COVID-19 vaccination. Please inform the investigator if you have received these vaccinations recently.
- You must not have had a significant infection within the 14 days prior to the challenge
- You must not smoke or use any tobacco products for the duration of the study.
- You must not use any prescription or non-prescription drugs and herbal supplements within 14 days prior to your challenge until the end of study. 2 grams of paracetamol (equivalent to 4 standard 500mg tablets or 3 sustained release 665mg tablets) per day and hormonal contraceptives are allowed, vitamins are prohibited within 7 days of your first follow up visit.
- You must not be related to anyone directly affiliated with this study.
- You must not use mouthwash from the day of screening until your first follow-up visit.
- You will not be permitted to consume any caffeine containing foods or beverages during your stay at the clinic. Decaffeinated coffee and tea may be provided to you.
- Report any changes in the way you are feeling to the study doctor or study staff at any point throughout the study.
- While you are resident within the study clinic you must maintain a good level of personal hygiene. This includes bathing daily (or when instructed by the study staff), bringing enough appropriate clean clothing with you for the duration of your stay, following staff instructions on cannula care and maintaining good hand hygiene (washing your hands after using the bathroom, before consuming your meals).

What effects could the tests have on me?

Blood Collection:

There is some slight discomfort involved in taking blood by indwelling cannula or with a needle and syringe. The insertion of a cannula is usually safe, but there are potential risks associated with this. There is a risk that a clot will form in the vein, which may take a number of weeks to resolve. There is a small risk of infection, although the use of sterile techniques and antiseptic solutions prior to insertion of the cannula minimises this risk. There is the possibility of bruising and discomfort around the site of an intravenous cannula, although this is generally minor and resolves within a few days. There is a very small risk that a nerve could be damaged during insertion of a cannula, however, the site of cannulation is carefully chosen to minimise this risk. Symptoms of nerve damage include tingling, shooting pain and pins and needles in the area of cannulation. Nerve damage may persist indefinitely, but usually resolves within 6-12 months. There is also the small chance that the cannula becomes dislodged from the vein, which can cause minor bleeding.

Procedures relating to blood collection can also occasionally cause light-headedness or fainting. These reactions are usually mild, of short duration and limited to a feeling of weakness, accompanied by sweating, slowing of heartbeat, and a decrease in blood pressure.

ECG:

As a result of the patches that are put on your skin when performing the ECG, there is the possibility a rash or minor irritation of the skin may result.

Midline Insertion:

Like insertion of a cannula for blood collection, the midline catheter insertion can cause some discomfort. This insertion is usually safe but there are some potential risks involved. Like cannula insertion there is a small risk of infection, although the use of sterile techniques and antiseptic solutions prior to insertion of the catheter minimises this risk. There is the possibility of bruising and discomfort around the insertion site but this normally resolves within days. There is a risk of inflammation of the vein (phlebitis) or swelling and redness in the tissue surrounding the catheter and there is a very small risk of the catheter moving out of place or breaking inside the vein, your midline will be inspected at least every 12 hours to ensure no complications have developed. Rarely a small or sometimes larger clot may form in the vein (thrombosis or deep vein thrombosis), if this is suspected the study doctor may refer you for additional tests or treatment.

There is a very small risk that a nerve could be damaged during insertion of a catheter, however, the site of cannulation is carefully chosen to minimise this risk. Symptoms of nerve

damage include tingling, shooting pain and pins and needles in the area of insertion. Nerve damage may persist indefinitely, but usually resolves within 6-12 months.

Throat Swabs:

You may experience some slight discomfort during the test and may gag a little when the swab is inserted.

Strep A Challenge:

Strep A has previously been used safely in a previous human challenge study conducted by members of this study's research team. 85% of participants in this study developed a sore throat after being exposed to Strep A and there were no serious side effects experienced by any participant over the course of the study.

You may develop symptoms such as a sore throat, fever, muscle aches or painful neck glands due to the administration of Strep A, however, the bacterial strain has been extensively tested and has previous been shown not to be associated with serious complications. This will minimise the risk of the complications, most of which are seen in untreated patients. Also, you will be given antibiotic treatment (azithromycin) as soon as the diagnosis criteria are met and therefore, your risk of developing complications relating to pharyngitis is very low. There is a very slight chance that you may experience a relapse of a sore throat following antibiotic treatment, you will be advised to contact the study doctor if you experience any further symptoms of pharyngitis and a study doctor will refer you for further treatment if confirmed.

If Strep A is left untreated and your immune system is unable to defend your body properly it can result in Invasive Group A Streptococcus infection (Invasive GAS infection). This is where the Strep A bacteria invades other parts of your body (e.g. your blood or lungs) and can cause a number of side effects, these can include a high fever, low blood pressure, vomiting, rashes and infection of the lung, bone or meninges (membranes that cover your brain). In rare cases patients can also develop a skin infection resulting in blisters, fever, fatigue and pain. As described earlier there is also the risk of ARF and RHD from untreated Strep A. There is also a risk of developing kidney inflammation as a result of the infection. As you will be receiving antibiotic treatment prior to discharge the risk of developing these side effects is extremely low, none of these severe side effects were seen in the previous Strep A study challenge in humans.

Sometimes people who experience a sore throat from Strep A become chronic asymptomatic carriers of Strep A, this means that you still have strep A bacteria in your throat but you show no symptoms. There is a small chance that this could happen to you following treatment. The study team will be checking for this by throat swab at each follow up visit but there is no evidence to show that being an asymptomatic carrier causes an increase in discomfort or transmission to others.

Strep A pharyngitis is considered a contagious illness. Due to this, precautions will be taken throughout your stay to prevent transmission to those around you. Strep A pharyngitis is not considered contagious 24 hours after antibiotic treatment, you should not be contagious on discharge.

What are the possible risks of the medication?

Benzylpenicillin has been used since the 1950s and is approved in Australia by the Therapeutic Goods Administration (TGA) as BENPEN™ for the treatment of a number of infections. BENPEN™ and other penicillins are generally well tolerated, side effects from BENPEN™ are very uncommon with 69% of side effects due to hypersensitivity (allergic reaction).

The following information provides a list of potential side effects which have been reported with the use of BENPEN™:

Common Side effects (Between 1 in 10 and 1 in 100 or 1-10% of people affected)

- Infusion site reaction (pain, redness, swelling)
- Abdominal pain
- Nausea
- Diarrhoea and Large Intestine Inflammation
- Allergy

Uncommon Side Effects (Between 1 in 100 and 1 in 1,000 or 0.1-1% of people are affected)

- Vomiting

Rare Side effects (between 1 in 1,000 and 1 in 10,000 or 0.01-0.1% of people are affected)

- Black Tongue
- High/Low levels of electrolytes
- Confusion
- Convulsions or seizures
- Coma Changes in blood test parameters

Side effects with Unknown Frequency

- Rash and Urticaria (hives – itchy, red skin rash)
- Fever
- Oedema (fluid build-up in tissue)
- Trouble breathing
- Hepatitis (liver inflammation) or other liver issues
- Abnormal kidney function

Other side effects which have been reported with the use of penicillin therapies such as BENPEN™ include anaphylaxis - a severe allergic reaction that can cause itchy rash, throat

swelling, a drop-in blood pressure and possibly death; and severe cutaneous adverse reactions (SCAR). SCAR is a rare but potentially fatal adverse drug reaction which may cause rash, fever, tissue death and damage to internal organs if left untreated. You will be closely monitored for any signs of anaphylaxis or SCAR and will not be able to participate in this study if you have a history of sensitivity to penicillin and some other allergens. The study doctor will discuss these in more detail with you.

Additional information related to the use of BENPEN™ will be provided to you in the Consumer Medicines Information (CMI) sheet.

There may be additional adverse effects in humans that are not yet known. If the study doctor has any concerns regarding your health during the study, additional tests may be performed to ensure your safety. These may include physical examinations, measuring your vital signs, performing an ECG and/or collecting blood samples to check your general health.

Linear's clinical facility is fully equipped with a crash cart (an emergency care trolley) and all staff are trained to deal with medical emergencies. In addition to appropriate first aid supportive measures by clinical and medical staff at Linear, your treatment may include the administration of various drugs, which may include adrenaline, anti-histamines or hydrocortisone. The local hospital Emergency Department will also be contacted if required.

Are there risks associated with becoming pregnant while participating in the study?

Previous human experience with penicillins during pregnancy has not shown any evidence of side effects on the foetus. Benzylpenicillin is classed as pregnancy Category A drug in Australia, meaning it has been taken by many pregnant women and women of child-bearing age without any proven increase in the frequency of malformations or other direct or indirect harmful effects on the foetus having been observed. There are, however, no adequate and well-controlled studies in pregnant women showing conclusively that harmful effects of these drugs on the foetus can be excluded.

It is recommended that participants use a condom for all sexual intercourse even if they or their partner are already on hormonal contraception for the entire duration of the study until completion of the follow-up visit. You should advise your study doctor if you become pregnant or father a child while participating in the research project. In the event you do fall pregnant or father a child within this period, the Sponsor may ask that you or your partner sign a separate consent form to allow monitoring of the pregnancy and the birth and the health of your child up to 1 year of age. Your study doctor will advise on medical attention for your partner should this be necessary.

It is highly recommended that you inform your partner of your participation in the study. You should advise your study doctor if you father a child while participating in the research project. Your study doctor will advise on medical attention for your partner should this be necessary.

You will be told in a timely manner about significant new information that might affect your decision to stay in the study.

What happens to my samples that are collected?

During the study, the estimated total blood volume to be collected will be approximately 160 mL (a little more than ½ a cup). As a reference, a standard blood donation is 470 mL in any 12-week period. You are advised not to donate any additional blood for 12 weeks after completing the study. As with all studies requiring blood donations, adequate rest and good eating habits are also advisable.

Your samples will be stored in either the laboratory of the Sponsor, or the laboratory of a company contracted to work with the Sponsor for a period of up to 7 years following the completion of the study. Access to study samples will be limited to laboratory personnel working for the Sponsor or who are contracted to work for the Sponsor and authorised to perform analyses. Your name will not be present on any samples and the individual performing the testing will not know your identity, solely the participant number that you are assigned will be present.

You have the right to withdraw from this study at any time, however, information and samples that already have been collected from you may continue to be used.

Additional information you need to know

Compensation for Injury

If you are injured as a result of your participation in this trial you may be entitled to compensation. There are two avenues that may be available to you to seek compensation.

1. Sponsors of clinical trials in Australia have agreed that the guidelines developed by their industry body, Medicines Australia, will govern the way in which compensation claims from injured participants are managed by Sponsors.

   However, as guidelines, they do NOT in any way dictate the pathway you should follow to seek compensation. The Sponsor is obliged to follow these guidelines.

   These guidelines are available for your inspection on the Medicines Australia Website (www.medicinesaustralia.com.au) under Policy – Clinical Trials – Indemnity & Compensation Guidelines. Alternatively, your study doctor can provide you with a hard-copy of the guidelines.

2. You may be able to seek compensation through the courts.

It is the recommendation of the independent ethics committee responsible for the review of this trial that you seek independent legal advice before taking any steps towards compensation for injury.

What benefits could there be from taking part in the study?

There will be no clear benefit to you from your participation in this research. Information learned from the study may help other people in the future.

Are there alternatives to participation?

Since this study is intended only to test the effect of the study drug in healthy volunteers, your alternative to being a volunteer in this study is to choose not to participate in the study.

Will I receive a fixed payment per visit to cover any out of pocket expenses?

The payment for participants who participate in and complete this study will be $2,695.

Participant payment is for your time and inconvenience. Your travel expenses and parking costs have been factored into this payment and will not be separately reimbursed.

A member of the study team will discuss with you the timing of the payments, including any interim payments you can expect.

Any payment received may be considered taxable income. Participants are encouraged to seek independent financial advice as to how any payment may affect your personal financial situation.

If you choose to withdraw your consent or in the unlikely scenario you are no longer required to participate in the study, then the level of payment you will receive will be on a pro-rata basis (i.e., you will receive a partial payment). You should also be aware that your study payment may be reduced or forfeited if you fail to follow any of the restrictions specified in this Participant Information Sheet. You will receive partial payment if you are withdrawn from the study due to non-medical reasons. You will receive full payment if you are withdrawn from the study because of medical reasons or a medical event related to the study.

Payment may be withheld entirely in the event that you are withdrawn from the study due to unacceptable behaviour, such as causing distress or behaving aggressively towards study staff or other participants.

Voluntary participation / Withdrawal from the study

Your participation in this study is purely voluntary. You may refuse to take part or withdraw from this study at any time without penalty or loss of benefits to which you are otherwise entitled. You may be withdrawn from the study if the study doctor feels it is best for you or if you do not comply with the requirements of the study. The Sponsor or the study doctor can also stop this study at any time for clinical or administrative reasons without regards to the participant consent.

If you wish to withdraw from the study while staying in the clinical trial unit, please discuss this with the study staff who will assist you in this matter and ensure you are medically fit to

leave. Before you leave the study, the study doctor will want to examine you, measure your vital signs and/or collect blood samples to check your general health.

Any significant new findings developed during the course of the research, which may affect your willingness to continue participation in this study, will be provided to you in a timely manner and may require you to sign an updated informed consent form in order to continue participating in the study.

Termination of the study

The research project may be stopped for a variety of reasons. These may include the following: unacceptable side effects, the drug being shown not to be effective, the drug being shown to work and not need further investigation, and decisions made in the commercial interest of the Sponsor.

How will my privacy be protected?

If you decide to be in this study, the study doctor and research team will use health data about you to conduct this study, as described in this consent form. This may include your name, address, phone number, medical history, photographs, date of birth, and information from your study visits. With your permission, this health data may come from your family doctor or other health care workers.

Please note that Linear does not sell any medications to the public and Linear personnel would never offer to sell any products to you. All medication provided by Linear for the purpose of the trial will always be free of charge to you.

By signing this document, you agree to allow the research team to share health data about you with government agencies and ethics committees that oversee the research, the Sponsor, and those working for the Sponsor, which may include affiliates of the Sponsor located in your country or other countries. An affiliate of the Sponsor includes all companies directly or indirectly owned by Telethon Kids Institute. To make sure the study rules are followed, people who work for the Sponsor will be able to see all health data about you when they visit the study site.

This clinical trial requires study monitors (people working for or on behalf of the trial Sponsor) to visit the study centre to review the clinical trial data so they can verify that study procedures have been followed and the study data have been entered correctly in the study records. This process is called ‘data verification’. An important part of this data verification includes confirming in a timely manner that the parts of the clinical trial that relate to protecting patient safety and wellbeing are properly performed at the study centre. This process typically takes place during regular monitoring visits to Linear Clinical Research’s facilities.

The health data that is sent to the Sponsor and those working for the Sponsor will not identify

you by name. Instead, it may include your initials, date of birth and study visit dates. You will not be identified by name in any published reports about this study or in any other scientific publication or presentation. If you think that you were harmed from being in the study, the study team may also share health data about you with the Sponsor's insurer to resolve your claim.

The Sponsor and those working for the Sponsor, which may include affiliates of the Sponsor, may use the health data sent to them:

- to see if the study drug has any side effects;
- how much of the study drug gets into the bloodstream and how long it takes to get rid of it;
- how much of the study drug is required to prevent infection

For these uses, the Sponsor may share this health data with others involved in these activities, as long as they agree to only use the health data as described here. The Sponsor and those working for the Sponsor, which may include affiliates of the Sponsor, may transfer health data about you from your country to other countries where the privacy laws are not as strict. Once the research team shares health data about you with others, it may no longer be protected by privacy laws. However, in the case of health data that identifies you, or from which your identity may be ascertained, an entity subject to Australian privacy laws that has collected your information must take reasonable steps to ensure that an overseas recipient handles the information in accordance with any relevant Australian privacy principle (unless an exemption applies). If you have any questions about this, direct them to the Principal Investigator.

Your permission to use and share health data about you will not end. You may however take away your permission to use and share any future health data about you at any time by writing to the study doctor. If you do this, you will not be able to stay in this study. No new health data that identifies you will be gathered after that date. However, health data about you that has already been gathered may still be used and given to others as described in this form.

In most cases, you have the right to request access to personal information collected from you in connection with the study and discuss any information that you believe is incorrect.

Your records relating to this study and any other information received will be kept strictly confidential. However, staff participating in your care, the Sponsor and other agencies authorised by law, may inspect the records related to the study. In the event you are admitted to hospital as a result of an adverse event resulting from this study, your treating doctor may require access to your study records. Auditors or inspectors (people who oversee the research to ensure compliance) may access your study record as well as any relevant external medical records, as authorised by law, during the study or in the future. Such access may occur at the study centre or remotely through a Linear Clinical Research secure electronic platform.

Your identity will not be revealed and your confidentiality will be protected in any reviews and reports of this study that may be published. However, results may be suppressed for commercial reasons as the Sponsor of the project retains the rights to the data.

Linear Clinical Research has a privacy policy which can be viewed at https://www.linear.org.au/privacy-policy/clinical-trial-participants/. It contains information about the use and disclosure of personal information relating to participants, and what Linear Clinical Research is required to do to maintain confidentiality of information.

Independence of Linear Clinical Research

Linear Clinical Research is an independent institute and is not part of the North Metropolitan Health Service or its hospitals and health services, or the Government of Western Australia. The health professionals involved in the conduct of this clinical research do so in a private capacity and not as employees of Sir Charles Gairdner Hospital or the State.

Will information about this trial be included in a Registry Databank?

A description of this clinical trial will be available on http://www.anzctr.org.au as required by the Declaration of Helsinki and the Australian National Statement on Ethical Conduct in Human Research. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

Who do I call if I have questions about…

- **The study or I need to report a medical condition (e.g. injury or illness):** Speak to a study team member at Linear Clinical Research Ltd. at (08) 6382 5100.
- **I need to speak to a doctor urgently:** Doctors at Linear Clinical Research can be contacted 24 hours a day, for urgent advice or emergencies, on (08) 6382 5116.

  If you experience a medical emergency, you should seek medical assistance by contacting 000 or attending your nearest hospital Emergency Department.
- **Your rights as a participant in the study:** The Bellberry Human Research Ethics Committee has reviewed this study in accordance with the National Statement on Ethical Conduct in Human Research (2007) incorporating all updates. This Statement has been developed to protect the interests of people who agree to participate in human research studies. Should you wish to discuss the study or view a copy of their Complaint procedure with someone not directly involved, particularly in relation to matters concerning policies, information or complaints about the conduct of the study or your rights as a participant, you may contact the Operations Manager, Bellberry Limited on 08 8361 3222.

Informed Consent Quiz

This quiz is designed to review your understanding of the study so that we can be confident that you fully understand what is involved by your agreement to participate. Please make sure you have read the information booklet in full and asked the study team any questions you have. If you don't answer all the questions correctly, don't worry. We will discuss these questions with you to ensure that you fully understand the study before signing consent.

Participant Name: _____

Date __ __ / __ __ __ / __ __ __ __

Please clearly circle one answer for each question;

1. **The study involves volunteers being given bacteria:**
   A. In a drink
   B. Using a throat swab
   C. In a tablet
   D. By injection

2. **If you participate in this study, you are likely to get which of the following:**
   A. Acute Rheumatic Fever
   B. Malaria
   C. Rheumatic Heart Disease
   D. 'Strep throat' (also known as pharyngitis)
   E. All of the above

3. **Screening for this study includes which of the following?**
   A. Urine and blood tests (including an HIV test)
   B. Physical examination
   C. Heart ultrasound (also known as an echocardiogram)
   D. The researchers contacting your GP (should you have one)
   E. All of the above

4. **Participation in the study involves:**
   A. Staying overnight in a research ward for up to 6 nights
   B. Having intravenous lines ('drips') put into your arms
   C. Getting a constant infusion (either medicine or a salty water placebo) through the drip
   D. Not consuming beverages containing caffeine (decaf will be allowed) for the duration of stay at the research facility
   E. All of the above

5. **During this study, we will collect what kind of samples from you?:**
   A. Blood, sweat, tears, urine
   B. Blood, stools, urine, saliva, nasal lining fluid
   C. Nasal lining fluid, saliva, blood, throat swabs
   D. Blood, throat swabs, skin biopsies

6. **Which of the following statements is <u>FALSE?</u>:**
   A. I can withdraw from the study at any time without penalty or any effect on medical care I will receive in the future.
   B. If I withdraw from the study after I have been given the Strep A bacteria, I will get a course of antibiotics to take regardless of whether I develop a throat infection or not.
   C. Once I give consent to participate in the study, I must complete the confinement period and I cannot withdraw my consent unless there is an emergency or permission from the investigators.
   D. I am able to withdraw from the study at any time but I understand the investigators will try to contact me after I leave to check on how I am doing.

7. **What strep throat symptoms might you experience during this challenge study?**
   A. Sore throat
   B. Fever
   C. Muscles aches
   D. Painful neck glands
   E. All of the above

8. **If I develop a sore throat during the study and experience pain or discomfort:**
   A. I will have to do my best to put up with it in the interest of medical science and helping others
   B. I will be reviewed by a medical doctor and will be offered pain relief medications to help ease my symptoms
   C. I will be given medicine to gargle and soothe my throat
   D. I will be given pain relief only if it is deemed severe

9. **Which of the following statements is <u>TRUE</u>?**
   A. I am putting myself at significant risk of developing acute rheumatic fever or rheumatic heart disease by taking part in this study.
   B. The bacteria used in the study has not been tested in humans before.
   C. I am not putting myself at significant risk of developing acute rheumatic fever or rheumatic heart disease because I am healthy and will receive antibiotics to treat my sore throat as soon as infection becomes apparent or before I am discharged.
   D. This research is being done because the investigators are not sure if penicillin works for prevention of acute rheumatic fever in people who are at risk.

Score: ___/ 9

Where applicable, the Investigator/Senior Researcher confirms that:

- There has been an opportunity to discuss and clarify any incorrect responses elicited during this pre-consent quiz
- The participant has, after discussion, demonstrated adequate understanding of what is involved in the CHIPS study and the Investigator/Senior Researcher is satisfied that they are able to provide informed consent.

Investigator/Senior Researcher signature _____

Date __ __ / __ __ __ / __ __ __ __

PARTICIPANT INFORMED CONSENT FORM

Sponsor Protocol Number: 1111-1264-9535

Study Name:	Controlled human infection for penicillin against *Streptococcus pyogenes*: a double blinded, placebo controlled, randomised trial
Study Doctor:	Associate Professor Laurens Manning

- I have read and understood this Participant Information Sheet and Informed Consent Form.
- I am between 18 to 40 years of age (inclusive) and have been given enough time to consider my participation and asked for advice if necessary.
- I have been given the opportunity to have a member of my family or another person present while the study is explained to me.
- I have had the opportunity to ask questions and have received satisfactory answers.
- I understand that all information will be kept confidential and that the results will be used for scientific objectives.
- I authorise my research and medical records as they pertain to this study to be reviewed by the Sponsor, authorised representatives and other regulatory agencies as described in this consent form.
- I understand that my participation in this study is voluntary and that I am completely free to withdraw my consent or refuse to participate at any time without changing in any way the quality of care that I receive. If I decide to withdraw from the study, I agree that the information collected about me up to the point when I withdraw may continue to be processed.
- I understand that if I agree to leave the study for any reason, the study doctor may ask me to do some end-of-study tests.
- The nature and purpose of the research project and potential risks and discomforts associated with it have been explained to me. I understand them and agree to take part.
- I consent to my GP and /or treating specialist being notified of my participation in this study and of any clinically relevant information noted by the study doctor in the conduct of the study, and to be contacted to obtain information regarding my medical history during the study and in the future for the purposes outlined in this information sheet.
- I confirm that I will provide, to the best of my knowledge, a full and accurate medical and surgical history and details of any current medical conditions and medicines I am taking.
- I agree to additional tests being conducted during the study, as requested by a study doctor, if the doctor has any concerns in relation to my health whilst on the study.
- I acknowledge that Linear Clinical Research is an independent institute and is not part of the North Metropolitan Health Service or its hospitals and health services, or the Government of Western Australia. The health professionals involved in the conduct of this clinical research do so in a private capacity and not as employees of Sir Charles Gairdner Hospital or the State.
- I have been given a copy of the Participant Information Sheet. I am aware that I will receive a copy of this fully signed and dated Informed Consent Form.
- As a healthy volunteer, I freely consent to participate in this study.

Participant's Name (printed):					
Signature:		**Date:**	__ __ / __ __ __ /__ __ __ __	**Time*:**	____ : ____

Please document any questions that the participant raised below and how they were answered.

Declaration by Investigator/Senior Researcher: I have given a verbal explanation of the study, its procedures and risks, have addressed any questions asked by the participant, and I believe that the participant has understood my explanations.

Investigator/Senior Researcher's Name (printed):					
Signature:		**Date:**	__ __ / __ __ __ / __ __ __ __	**Time*:**	____ : ____

**** Participant must sign the consent form first, prior to the Investigator/Senior Researcher.***
Note: All parties signing the Consent Form must date their own signature.

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Page no.
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, name of intended registry	16
	2b	All items from the World Health Organization Trial Registration Data Set	16
Protocol version	3	Date and version identifier - *Not included in manuscript but text prepared based on HREC approved CHIPS trial protocol V1.0 Dated 16 April 2021*	
Funding	4	Sources and types of financial, material, and other support	18
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 18
	5b	Name and contact information for the trial sponsor	16
	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	18
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	14, Fig 3

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	6
	6b	Explanation for choice of comparators	6
Objectives	7	Specific objectives or hypotheses	9, Table 2
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, noninferiority, exploratory)	7
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 be obtained	7
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)	Supp
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	10
	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)	12
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	12
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	10
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	9, Table 2

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)	Fig 2
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	15
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	11
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	11
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	11
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	11
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	11
Methods: Data collection, management, and analysis			

Data collection methods	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	
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	12
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
Statistical methods	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	16
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	16
	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)	16
Methods: Monitoring			
Data monitoring	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 not needed	14, Fig 3
	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, Fig 3

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
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	14
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	16
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)	16
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	10
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable - *Not included in the manuscript but specified in the HREC approved study protocol*	
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	14
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	18
Access to data	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	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
	31b	Authorship eligibility guidelines and any intended use of professional writers - *Not included in the manuscript but specified in the HREC approved study protocol*	
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code - *Not included in the manuscript but specified in the HREC approved study protocol*	
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates - *Not included in the manuscript but specified in the HREC approved study protocol*	
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 - *Not included in the manuscript but specified in the HREC approved study protocol*	

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.