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Bordetella Pertussis colonisation: design of a B. pertussis human challenge colonisation model for biomarker identification and vaccine development.

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3 ***Bordetella Pertussis* colonisation: design of a *B. pertussis* human challenge colonisation model for**
4 **biomarker identification and vaccine development.**
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Abstract:

INTRODUCTION: We summarise an ethically approved protocol for the development of an experimental *Bordetella pertussis* human challenge colonisation model. Globally *B. pertussis* is one of the leading causes of vaccine preventable death. Many countries have replaced whole cell vaccines with acellular vaccines over the last 20 years during which pertussis appears to be resurgent in a number of countries in the developed world that boast high immunization coverage. The acellular vaccine provides relatively short-lived immunity and, in contrast to whole cell vaccines, may be less effective against colonisation and subsequent transmission. To improve vaccine strategies, a greater understanding of human *B. pertussis* colonisation is required.

METHODS AND ANALYSIS: A controlled human colonisation model will be developed over two phases. In phase A, a low dose of the inoculum will be given intranasally to healthy participants. This dose will be escalated or de-escalated until a colonisation rate of approximately 70% is reached without causing disease. The colonisation period, transmission and exploratory immunology will be assessed during a 17 day inpatient stay and follow-up over one year. The dose of inoculum which achieves 70% colonisation will then be confirmed in phase B, comparing healthy participants exposed to *B. pertussis* with a control group receiving a sham inoculum.

ETHICS AND DISSEMINATION: REC reference: 17/SC/0006, 24 February 2017. Findings will be published in peer reviewed open access journals as soon as possible.

Strengths and limitations of this study:

- A controlled human colonisation model with *Bordetella pertussis* will provide greater understanding of *B. pertussis* colonisation, required for future vaccine development.
- The model will give insight in the interaction between host and *B. pertussis* during colonisation.
- Controlled challenge might not reflect natural colonisation accurately.
- The individual variation and the low number of participants might influence the external validity of the results
- Aiming for 70% colonisation may be suboptimal for future vaccine efficacy studies using this model.

Introduction:

Pertussis, also called whooping cough, is an acute bacterial infection caused by *Bordetella pertussis* (Bp), an exclusively human pathogen. Although it can affect people of all ages, young unimmunised infants are the most vulnerable group with the highest rates of complications and death (1). In 1999 there were an estimated 48.5 million pertussis cases in children worldwide and 295,000 deaths (2), but based on sero-epidemiological prevalence studies the number of asymptomatic Bp infections may be much higher (3). By 2013, mortality fell to about 60,600 (interquartile range 22,300-136,800) children per year, still making it one of the leading causes of vaccine-preventable death (4).

Pertussis vaccines have been included in National Immunisation Programmes since their introduction in the 1940s-1950s. Acellular pertussis vaccines (aP) have a favourable reactogenicity profile in comparison to whole-cell pertussis vaccines (wP) and are currently mostly used in industrialised countries to immunise against pertussis (5). However, many countries in the developed world that boast high immunization coverage have seen an increase in the incidence of pertussis over the past 20 years (6). Five main hypotheses have been proposed to contribute to this resurgence: 1) rapid waning of immunity following vaccination, especially with aP (7) 2) the very different immune response profile induced by aP compared to wP vaccines (8), 3) adaptation of Bp to escape protective immunity, 4) low vaccine coverage and 5) less effective reduction of transmission from infected individuals vaccinated with aPs (9). The latter is supported by baboon studies that have demonstrated that vaccination with aP prevents severe disease but does not prevent asymptomatic infection, i.e. colonisation (10). Studies in mice are consistent with these findings and demonstrate that protective immunity is more effective and persistent when induced by natural infection or wP than by aP (11). To study the pathogenesis of pertussis a variety of animal models have been used, including mice, rabbits, guinea pigs, and newborn piglets (12). However, there are still important knowledge gaps relating to human immunity to Bp and it is not clear to what extent these observations in animal models translate into clinical practice. This paucity of knowledge hampers the development of improved vaccines and the design of better vaccination strategies against pertussis in infants, adolescents and adults.

The deliberate infection of human volunteers with micro-organisms has contributed uniquely to our understanding of the pathogenesis, immune responses and the treatment and prevention of numerous microbial diseases including pneumococcal disease (13), influenza, cholera, typhoid and hepatitis (14). We aim to develop a safe human challenge **colonisation** model to get a more thorough understanding of the immune response against wild type Bp and to facilitate development of bioassays and next generation pertussis vaccines.

Methods:

This is a controlled human infection study consisting of two phases; phase A: development of a Bp human challenge colonisation model and phase B: Development of a modified Bp human challenge colonisation model in which we will compare participants receiving the standard inoculum (SI) with a control group receiving a sham inoculum. This article summarises the protocol for phase A.

Phase A will be a dose finding phase in which the dose of inoculum will be escalated or de-escalated to find the Standard Inoculum (SI), defined as the dose of Bp that will safely cause colonisation in approximately 70% of the participants. The dose escalation / de-escalation process designed to find the SI is shown in figures 1-3. Phase A will be finished once a total of 10 participants will have been successfully colonised with the SI.

Ethics and dissemination:

In the UK and EU, an experimental human challenge study with a wild-type bacterium falls outside the European Clinical Trials Directive (14). Participants in challenge trials are healthy volunteers who do not obtain direct health benefit from participation. Potential volunteers will be informed of all conceivable risks and have adequate time to decide on their individual participation in relation to the risks involved and financial compensation for the time and inconvenience of taking part. Medical ethicists have argued that it is a healthy adult's right to self-determine their participation in such trials in relation to the risk (Controlled Human Infection Studies in the Development of Vaccines & Therapeutics Wellcome Trust Scientific Conference, January 9-11, 2013, University of Cambridge, UK).

The protocol has been reviewed by independent peer reviewers including experts from Public Health England, and worldwide experts on pertussis within the Periscope consortium (www.periscope-project.eu) who have assessed the safety and the quality of the study, the study design and the feasibility of the study objectives. The protocol has been reviewed and approved by the South Central - Oxford A Research Ethics Committee (REC reference: 17/SC/0006, 24 February 2017) and the UK Health Research Authority (IRAS project ID: 219496, 1/3/2017). Findings will be published in peer reviewed open access journals as soon as possible. The final protocol for phase B will be presented as a substantial protocol amendment, because it will be based on the standard inoculum and colonisation period identified in phase A as well as the outcome of the exploratory immune assays performed during this phase.

Study objectives:

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3 The primary objective of phase A of this study is to determine the dose of the standard inoculum in
4 participants who do not have evidence of recent Bp exposure. The standard inoculum is defined as
5 the dose of Bp causing colonisation and seroconversion in approximately 70% of the participants
6 between day 0 and 14 without causing Bp disease after being challenged with this dose at day 0. An
7 endpoint of 70% is used to avoid, if possible, a `saturating dose` that results in non-physiological
8 colonisation by Bp in participants, but still induces sufficiently high colonisation rates to enable
9 potential future vaccine efficacy trials.. Secondary endpoints will explore the ability to dose the
10 inoculum accurately, the pre- and post-challenge Bp-specific immunity in healthy participants and
11 the environmental shedding of Bp following nasal inoculation (see table 1). Feedback and
12 recommendations from Patient and Public Involvement (PPI), in addition to participant
13 questionnaires from previous studies, have been incorporated into this study.

21 *Challenge strain*

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24 The Bp isolate to be used in this human colonisation model is B1917, which is representative of
25 current isolates in Europe (15). The strain, isolated in 2000 from a Dutch patient with Bp disease, is
26 characterised as *ptxP3-ptxA1-prn2-fim3-2, fim2-1* MLVA27, PFGE BpSR11 and expresses pertactin
27 (PRN), pertussis toxin (PT) and filamentous hemagglutinin (FHA). This strain has been extensively
28 characterised in the mouse model as well as by proteomics and transcriptomics and has a closed
29 genome available (15). It is fully sensitive to azithromycin *in vitro*.

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34 The inoculum has been prepared by Q Biologicals (Ghent, Belgium) according to good manufacturing
35 practice (GMP) standard in licensed GMP facilities and using a process free of animal-derived
36 products. The identity and purity of the cell bank have been confirmed, in addition to any other
37 quality specifications agreed within the consortium and needed for compliance with regulatory
38 requirements. Before intranasal inoculation, there will be no further culture of the challenge
39 inoculum at the clinical site. The dose and purity of the inoculum will be determined after
40 inoculation for quality assessment.

46 *Study setting*

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49 This is one of several other clinical and preclinical studies of the Periscope consortium
50 (<http://periscope-project.eu/consortium/>) which brings together internationally renowned scientists
51 with many years of experience in Bp research, clinical trials, bioinformatics, immunology and public
52 health. The aim of the consortium is to promote scientific and technological innovation in pertussis
53 vaccine development and to foster the creation of a laboratory and scientific network that facilitates
54 the testing and helps expedite the development of novel pertussis vaccines in Europe.

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3 The study will be conducted by the University of Southampton in the National Health Institute for
4 Health Research Clinical Research Facility (CRF) Southampton.
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7 *Recruitment*

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9 Participants will be recruited via the following:

- 11 • Southampton CRF and Periscope websites – information about the study will be available on
12 the website with a downloadable volunteer information sheet
- 13 • Posters in public places, including buses and trains, university campus, student bars, halls of
14 residence, health centres etc. with the agreement of the owner / proprietor
- 15 • Newspapers or other literature for circulation
- 16 • Press release
- 17 • Clinical Research Update magazine
- 18 • A post on a Twitter, Facebook or Gumtree account owned and operated by our group
- 19 • Email distribution to individuals who have already expressed an interest in taking part in any
20 clinical trial at the CRF Southampton.
- 21 • Southampton CRF Database of Healthy Volunteers: individuals from this database have
22 previously expressed an interest in receiving information about future studies for which they
23 may be eligible.

24
25 Potential volunteers who are interested will be sent the volunteer information sheet and will be
26 invited for a screening visit. During this visit they will be given an opportunity to discuss the study
27 questions and complete a pre-consent questionnaire to ensure they understand the study. The
28 informed consent procedure includes specific infection prevention information regarding the
29 measures that are taken to prevent transmission during admission to the research unit. Once
30 consent has been given, their eligibility will be assessed including a general health questionnaire.
31 The medical history will be checked with the general practitioner. Participants will be offered
32 reimbursement for their time, travel and inconvenience.
33

34 *Eligibility criteria*

35
36 Healthy volunteers, men and women, aged 18-45 without known or suspected recent pertussis
37 infection (anti-PT IgG >20 IU/mL) will be recruited for phase A. Care will be taken not to recruit from
38 vulnerable groups (mental health or other impaired capacity issues). Specific inclusion and exclusion
39 criteria can be found in the online supplementary Table 1. These criteria should minimise the risk of
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3 complicated disease or transmission of Bp to risk groups and aim to select a homogenous group that
4 will be able to adhere to the scheduled admission and follow up visits.
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7 *Interventions*

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9 The start of volunteer participation is defined as the screening visit. The end of volunteer
10 participation is defined as the last visit. The duration of involvement in this study from screening will
11 be approximately 56 weeks. A detailed schedule of the interventions is shown in table 2.
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15 A week before the challenge nasal samples will be collected. On the day of the challenge the
16 participant will be admitted to a designated area in the CRF and will have access to a dedicated
17 individual bedroom, shared toilet, shower and recreational areas during their stay in the facility.
18 Participants will be allowed to leave the designated area during the daytime for a maximum of two
19 hours twice a day. Participants choosing to leave the CRF will be asked to wear a face mask while
20 inside hospital buildings to ensure no transmission. When they leave or come back to the designated
21 area, they will be escorted by a member of the study team. The Bp inoculum will be prepared and
22 administered following study-specific standard operating procedures (SOPs) which are based on
23 previous human challenge studies using nasal inoculation with *N. lactamica* (16) (17). One vial of
24 inoculum will be removed from the freezer, thawed and diluted to the required inoculum dose. The
25 participant will be positioned supine with neck extended, mouth open, and breathing normally
26 through their mouth. 0.5 mL of the inoculum will be gently expelled into each nostril, while the
27 participant is positioned with mouth open, neck extended and breathing normally through their
28 mouth. After the inoculum is administered the vial residuum will be diluted and cultured for 5 days
29 on *Bordetella* selective medium for determination and viable counts of Bp and on non-selective
30 medium to assess purity of the inoculum.
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42 During admission, clinical observations and symptoms of possible early pertussis disease such as
43 rhinorrhoea, nasal congestion, epistaxis, sneezing, ear pain, eye pain, sore throat, cough, dyspnoea,
44 feeling generally unwell, tiredness and headache will be reviewed six times per day. Regular nasal,
45 mouth, throat and blood samples will be taken to assess the microbial dynamics and the host
46 response to the challenge. Environmental samples will be taken to assess transmission. These
47 include cultures and quantitative polymerase chain reaction (qPCR) of: face masks, contact areas in
48 the personal room, air sampling of the personal room and during aerosol producing activities.
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54 Colonisation will be defined as a positive culture of Bp from the nasopharyngeal swab specimen.
55 After 5 participants have received the inoculum, the colonisation rate will be assessed and the
56 following 5 participants will receive either the same dose or an escalated (Figure 2) or decreased
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3 (Figure 3) dose with the aim of achieving colonisation in 70% of the challenged volunteers. The
4 standard inoculum will continue to be administered until 10 participants have been colonised at that
5 dose. The various samples (nasopharyngeal swab, throat swab, nasal wash, and nasal fluid sample)
6 will be compared to identify the technique that yields the highest sensitivity of Bp detection to
7 inform the design of Phase B. The seroconversion rate will be measured comparing pre- and post-
8 inoculation serum samples of participants infected after receiving the standard inoculum.
9 Seroconversion will be defined as a 3-fold rise in anti-PT IgG titre (IU/mL) from day 0 to day 28. We
10 will then identify the 'colonisation period' which is defined as the earliest day after inoculation on
11 which colonisation of the nasopharynx (as detected by the most sensitive technique) is observed in
12 100% of those volunteers who subsequently seroconvert at day 28. The colonisation period will be
13 used in the protocol for Phase B to minimise the duration of infection prior to eradication of Bp by
14 antibiotics.

15
16 In Phase A, azithromycin 500 mg will be given once a day for three days to eradicate possible Bp
17 colonisation on day 14 or to treat possible early stage Bp disease before day 14 following national
18 guidelines (18). After treatment, participants will remain in the hospital for another 48 hours to
19 assess eradication success and environmental shedding after eradication. If eradication is
20 unsuccessful 48 hours after eradication the course of azithromycin will be repeated. Follow up visits
21 will take place at week 4, 8, 26 and 52.

22
23 If, following inoculation, the participant develops symptoms of early pertussis disease, azithromycin
24 will be commenced according to Public Health England treatment guidelines. This will be done
25 following a pre-defined treatment algorithm (see Figure 4) which is designed to exclude trivial viral
26 infection or transient and non-specific upper respiratory tract symptoms. After treatment the
27 participant will continue to be included in the study and specimens will be collected as if they had
28 received eradication treatment at day 14 per protocol.

29 *Safety*

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31 Our priority is to develop this model without causing harm to the individual or the environment. An
32 important facet of a safe model will be to limit participant exposure to Bp to the minimum dose and
33 number of days required to signal successful colonisation. Safety considerations in the protocol
34 include:

- 35 1. The dose of the inoculum will start low and will be modified to the lowest dose required to
36 effectively establish colonisation with regular reviews of the available safety data by the
37 study steering committee and the external safety committee.

2. The timing of eradication will be modified to optimise the safety of the participants by exposing them to the minimal effective period of colonisation.
3. The incubation time for the catarrhal phase is unknown, but is estimated to be 7 – 22 days. After inoculation of the participants, there will therefore be a moderate risk of symptom development because eradication therapy will initially be given at day 14.
4. If a participant shows signs of possible disease there will be a low threshold to eradicate colonisation.
5. Bp disease in adults is often atypical and relatively mild. Treatment is not thought to reduce the duration of coughing in natural infection, but adults with natural infection will not usually receive treatment until a late stage of the disease, if at all. Early treatment (as proposed in this study) is considered more likely to reduce symptoms quickly
6. The likelihood of severe disease is extremely low and the risk of harm to the participants, staff and others can be minimised by admitting the participants as inpatients in the Southampton CRF which is a NIHR facility specifically funded to conduct higher risk experimental medicine in a safe National Health Service (NHS) environment. Household contacts will not be asked to provide informed consent, but volunteers with household members within the risk groups for pertussis disease will be excluded from the study.
7. All health care workers involved in this study will be vaccinated against Bp at least 2 weeks before working with participants that have received the inoculum unless they have been vaccinated against Bp in the last 5 years (19).

An external safety committee of independent infectious disease experts will assess the safety of study after every 5 participants and advise on the continuation of the study.

Sample size

Phase A is a dose finding study and the sample size has been estimated with the assumption that we will colonise no, or only a few, participants at the initial low dose, allowing for escalation of the dose based on colonisation rate and safety parameters. We are aiming to achieve colonisation and seroconversion in 10 participants with the standard inoculum dose of Bp.

Statistical analysis

Formal statistical comparisons of colonisation/ seroconversion will not be carried out in phase A. In this dose finding phase, decisions to escalate or de-escalate the dose will be based on the results accumulating over the progression of the study.

Discussion

To the best of our knowledge this will be the first adult human challenge study with wild type Bp. A paediatric Bp human challenge study was performed in 1933 in which two naive and two vaccinated children were exposed to 140 cfu of intranasal Bp (20). While the vaccinated children did not develop whooping cough the naive children developed severe disease including anorexia, fever, severe paroxysmal cough, leucocytosis, lymphocytosis and positive cultures. They received no treatment and improved by day 35. This study confirmed that pertussis disease is caused by the bacillus of Bordet and Gengou and could be prevented by the experimental Bp vaccine that was used.

In a recent first in human study, healthy volunteers were given a live attenuated Bp strain as a nasal vaccine (21). The inoculated strain was genetically modified; dermonecrotic toxin and tracheal cytotoxin were removed, and PT was genetically detoxified by two independent mutations, removing the toxic activity of PT without affecting its immunogenic properties. Three groups, each of 12 participants, were inoculated with 10^3 , 10^5 or 10^7 colony forming units respectively. Colonisation was seen in one subject in the low dose, one in the medium dose and five in the high dose group. Adverse events occurred in similar frequency in all groups, including the placebo group and were found to be trivial. The effect of the genetic changes on the ability to colonise are unknown but it is expected to have a significant effect on colonisation.

The inoculum required to colonise previously vaccinated adults exposed to natural infection with Bp is unknown, but is assumed to be higher than that used in the paediatric challenge study. The initial inoculum dose used in our study (1000 cfu) has been chosen as a dose low enough to be assumed to be safe, but high enough to allow accurate monitoring.

This model presumes that Bp disease is preceded by colonisation. Direct evidence for this has proven hard to obtain in surveillance studies (22, 23). Potential reasons for this are the use of inadequate sampling methods or insufficiently sensitive assays, or that colonisation is a very short phenomenon and often missed in a cross-sectional study. Seroprevalence studies do show there is a natural boosting of anti-PT IgG levels, suggesting asymptomatic infection/colonisation is quite frequent (24-26).

To minimise the risk for the participant this controlled infection study is not a disease model, as was the case with many other challenge studies (27) (28), but a colonisation model. The risk of symptom development in Phase A is moderate because eradication therapy is not planned to be given until day 14 to allow an adequate immune response to develop. Eradication therapy will be given earlier if

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3 symptoms develop that are suggestive of pertussis disease. This approach is considered to be
4 acceptable because the risk of severe disease in healthy adults is extremely low (29, 30). Antibiotic
5 treatment is known to be effective at eradicating Bp if given early after diagnosis, but does not alter
6 the subsequent clinical course of the illness (31), especially if administered beyond 2–3 weeks after
7 the onset of symptoms. In this study treatment will be given at an early stage of the infection, which
8 we predict will have a more positive effect on the disease course.
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13 We have implemented comprehensive transmission prevention measures, including an in-patient
14 stay of 17 days. The transmission of Bp, the colonisation rate and colonisation period will be
15 evaluated during phase A alongside the host immune response, to inform a more directed approach
16 in phase B.
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21 The target colonisation rate of 70% was chosen partly to be appropriate for future studies requiring
22 a comparison of colonised participants with non-colonised/protected participants, for example
23 studies aiming to identify protective biomarkers. This colonisation rate might not be appropriate for
24 studies designed to assess the efficacy of novel vaccines in prevention of colonisation where a near
25 100% colonisation rate would be preferable. Therefore the standard inoculum dose may be
26 increased in future studies, depending on the results of this initial study.
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31 We recognise that this method of intranasal inoculation to induce colonisation / infection differs
32 from the natural course of infection. However, this model will provide the opportunity to study the
33 systemic and local immune response to exposure to Bp in a way that would not be feasible in natural
34 human exposure or accurately represented by an animal model of exposure. Because of the atypical
35 and often late presentation of pertussis in adults, knowledge about the features of pre-symptomatic
36 and early infection is lacking. This study will give a unique insight in the initial interaction between
37 bacteria and host during the first two weeks after initial exposure. The development of a safe human
38 challenge model of pertussis, in conjunction with the recently developed baboon model of pertussis,
39 has the potential to provide a path forward for answering critical questions about pertussis
40 pathogenesis and host responses and will likely aid in the development of next-generation pertussis
41 vaccines (32). Within the Periscope consortium we aim to compare the results of this study with
42 animal studies, vaccination studies with acellular and whole cell vaccines and natural infection
43 studies with Bp.
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3 *Full references*
4

- 5 1. England PH. Factsheet Pertussis: Public Health England; 2016 [Available from:
6 [https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/323000/Factsheet](https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/323000/Factsheet_for_healthcare_professionals.pdf)
7 [_for_healthcare_professionals.pdf](https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/323000/Factsheet_for_healthcare_professionals.pdf).
8
- 9 2. Crowcroft NS, Stein C, Duclos P, Birmingham M. How best to estimate the global burden of
10 pertussis? *The Lancet Infectious diseases*. 2003;3(7):413-8.
- 11 3. de Greeff SC, Mooi FR, Westerhof A, Verbakel JM, Peeters MF, Heuvelman CJ, et al. Pertussis
12 disease burden in the household: how to protect young infants. *Clinical infectious diseases : an*
13 *official publication of the Infectious Diseases Society of America*. 2010;50(10):1339-45.
- 14 4. Collaborators GMAcOD. Global, regional, and national age-sex specific all-cause and cause-
15 specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of
16 Disease Study 2013. *Lancet (London, England)*. 2015;385(9963):117-71.
- 17 5. Zhang L, Prietsch SO, Axelsson I, Halperin SA. Acellular vaccines for preventing whooping
18 cough in children. *The Cochrane database of systematic reviews*. 2014(9):Cd001478.
- 19 6. Jackson DW, Rohani P. Perplexities of pertussis: recent global epidemiological trends and
20 their potential causes. *Epidemiology and infection*. 2014;142(4):672-84.
- 21 7. Klein NP, Bartlett J, Rowhani-Rahbar A, Fireman B, Baxter R. Waning protection after fifth
22 dose of acellular pertussis vaccine in children. *The New England journal of medicine*.
23 2012;367(11):1012-9.
- 24 8. Brummelman J, Wilk MM, Han WGH, van Els CACM, Mills KHG. Roads to the development of
25 improved pertussis vaccines paved by immunology. *Pathogens and disease*. 2015;73(8):ftv067.
- 26 9. Warfel JM, Zimmerman LI, Merkel TJ. Acellular pertussis vaccines protect against disease but
27 fail to prevent infection and transmission in a nonhuman primate model. *Proceedings of the*
28 *National Academy of Sciences of the United States of America*. 2014;111(2):787-92.
- 29 10. Warfel JM, Papin JF, Wolf RF, Zimmerman LI, Merkel TJ. Maternal and neonatal vaccination
30 protects newborn baboons from pertussis infection. *The Journal of infectious diseases*.
31 2014;210(4):604-10.
- 32 11. Higgs R, Higgins SC, Ross PJ, Mills KH. Immunity to the respiratory pathogen *Bordetella*
33 *pertussis*. *Mucosal immunology*. 2012;5(5):485-500.
- 34 12. Elahi S, Holmstrom J, Gerds V. The benefits of using diverse animal models for studying
35 *pertussis*. *Trends in microbiology*. 2007;15(10):462-8.
- 36 13. Ferreira DM, Jambo KC, Gordon SB. Experimental human pneumococcal carriage models for
37 vaccine research. *Trends in microbiology*. 2011;19(9):464-70.
- 38 14. Sciences AoM. *Microbial Challenge Studies of Human Volunteers*. 2005.
- 39 15. Bart MJ, van der Heide HG, Zeddeman A, Heuvelman K, van Gent M, Mooi FR. Complete
40 Genome Sequences of 11 *Bordetella pertussis* Strains Representing the Pandemic ptxP3 Lineage.
41 *Genome announcements*. 2015;3(6).
- 42 16. Deasy A, Guccione E, Dale A, Andrews N, Evans C, Bennett J, et al. Carriage of the commensal
43 *Neisseria lactamica* inhibits acquisition and carriage of *Neisseria meningitidis* in the nasopharynx.
44 *Association of Physicians Annual Meeting, Belfast 2013*. 2013.
- 45 17. Evans CM, Pratt CB, Matheson M, Vaughan TE, Findlow J, Borrow R, et al. Nasopharyngeal
46 colonization by *Neisseria lactamica* and induction of protective immunity against *Neisseria*
47 *meningitidis*. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of*
48 *America*. 2011;52(1):70-7.
- 49 18. England PH. Guidelines for the Public Health Management of Pertussis in England
50 London2016 [Available from:
51 [https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/541694/Guideline](https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/541694/Guidelines_for_the_Public_Health_Management_of_Pertussis_in_England.pdf)
52 [s_for_the_Public_Health_Management_of_Pertussis_in_England.pdf](https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/541694/Guidelines_for_the_Public_Health_Management_of_Pertussis_in_England.pdf).
53
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19. England PH. Guidance Whooping cough (pertussis): immunisation of healthcare workers 2013 [Available from: <https://www.gov.uk/guidance/whooping-cough-pertussis-immunisation-of-healthcare-workers>].
20. MacDonald H, MacDonald EJ. Experimental pertussis. *The Journal of infectious diseases*. 1933;53(3):328-30.
21. Thorstensson R, Trollfors B, Al-Tawil N, Jahnmatz M, Bergstrom J, Ljungman M, et al. A phase I clinical study of a live attenuated *Bordetella pertussis* vaccine--BPZE1; a single centre, double-blind, placebo-controlled, dose-escalating study of BPZE1 given intranasally to healthy adult male volunteers. *PloS one*. 2014;9(1):e83449.
22. Linnemann CC, Jr., Bass JW, Smith MH. The carrier state in pertussis. *American journal of epidemiology*. 1968;88(3):422-7.
23. Zhang Q, Yin Z, Li Y, Luo H, Shao Z, Gao Y, et al. Prevalence of asymptomatic *Bordetella pertussis* and *Bordetella parapertussis* infections among school children in China as determined by pooled real-time PCR: a cross-sectional study. *Scandinavian journal of infectious diseases*. 2014;46(4):280-7.
24. Mertsola J, Ruuskanen O, Eerola E, Viljanen MK. Intrafamilial spread of pertussis. *The Journal of pediatrics*. 1983;103(3):359-63.
25. Huygen K, Rodeghiero C, Govaerts D, Leroux-Roels I, Melin P, Reynders M, et al. *Bordetella pertussis* seroprevalence in Belgian adults aged 20-39 years, 2012. *Epidemiology and infection*. 2014;142(4):724-8.
26. He H, Yao P, Zhou Y, Deng X, Pan J. Is Pertussis Infection Neglected in China? Evidence from a Seroepidemiology Survey in Zhejiang, an Eastern Province of China. *PloS one*. 2016;11(5):e0155965.
27. McCullagh D, Dobinson HC, Darton T, Campbell D, Jones C, Snape M, et al. Understanding paratyphoid infection: study protocol for the development of a human model of *Salmonella enterica* serovar Paratyphi A challenge in healthy adult volunteers. *BMJ open*. 2015;5(6):e007481.
28. Rampling T, Ewer KJ, Bowyer G, Bliss CM, Edwards NJ, Wright D, et al. Safety and High Level Efficacy of the Combination Malaria Vaccine Regimen of RTS,S/AS01B With Chimpanzee Adenovirus 63 and Modified Vaccinia Ankara Vectored Vaccines Expressing ME-TRAP. *The Journal of infectious diseases*. 2016;214(5):772-81.
29. Farizo KM, Cochi SL, Zell ER, Brink EW, Wassilak SG, Patriarca PA. Epidemiological features of pertussis in the United States, 1980-1989. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 1992;14(3):708-19.
30. De Serres G, Shadmani R, Duval B, Boulianne N, Dery P, Douville Fradet M, et al. Morbidity of pertussis in adolescents and adults. *The Journal of infectious diseases*. 2000;182(1):174-9.
31. Altunajji S, Kukuruzovic R, Curtis N, Massie J. Antibiotics for whooping cough (pertussis). *The Cochrane database of systematic reviews*. 2007(3):CD004404.
32. Merkel TJ, Halperin SA. Nonhuman primate and human challenge models of pertussis. *The Journal of infectious diseases*. 2014;209 Suppl 1:S20-3.

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5 H. de Graaf, has written the protocol
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10 Andrew Gorringe, assisted and advised writing the protocol
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12 Dimitri Diavatopoulos, assisted and advised writing the protocol
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15 Kent Kester, assisted and advised writing the protocol
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18 Saul N. Faust, assisted and advised writing the protocol
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20 Robert C. Read, has written the protocol
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30 Industries and Associations (EFPIA) and Bill and Melinda Gates Foundation (BMGF).
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34
35 *Competing interests statement:*
36

37 Hans de Graaf has received sponsorship from Abbvie to attend a clinical paediatric rheumatology
38 course
39

40
41 Saul N. Faust acts on behalf of the University of Southampton/University Hospital Southampton NHS
42 Foundation trust as chief and principal investigator for clinical trials Sponsored by vaccine and
43 antimicrobial manufacturers but receives no personal payments for the work.
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Table 1: Objectives and endpoints of the study

	Objectives	Endpoints
1A	To determine the dose of the standard inoculum in participants who do not have evidence of recent Bp exposure - Safety	Safety endpoints: - Occurrence of possible or confirmed Bp disease within the study period - Occurrence of unsolicited adverse events within the study period - Occurrence of serious adverse events within the study period
1B	To determine the dose of the standard inoculum in participants who do not have evidence of recent Bp exposure – 70% colonisation	Microbiologically proven Bp colonisation by positive culture of Bp from a nasopharyngeal swab taken between time points day 0 and 14 after being challenged on day 0
2	To evaluate accuracy of the inoculum dosing	Estimation of the actual challenge dose in comparison to the prescribed challenge dose by viable count (cfu/mL) of residual inoculum following inoculation of each participant
3	To describe the human physiological response to Bp challenge in those developing or not developing infection	Description of the clinical course after challenge using, for example: Clinical and laboratory observations such as temperature (°C), systolic blood pressure (mm Hg), diastolic blood pressure (mm Hg), heart rate (beats/min), respiratory rate (breaths/min), O ₂ saturation in blood (%), CRP (mg/L), WBC (10 ⁹ /L), Lymphocyte count (10 ⁹ /L) at various time-points
4	To determine the colonisation period: the earliest day after inoculation at which colonisation of the nasopharynx (as detected by culture) is observed in 100% of those participants who subsequently seroconvert at day 28	A 3-fold rise in anti-PT IgG titre (IU/mL) from day 0 to day 28 will be used as a marker of seroconversion. Colonisation will be detected by positive culture of Bp from a nasopharyngeal swab taken between time-points day 0 and 14
5	To determine the characteristics of bacterial dynamics after challenge	Microbiological assays to detect and characterise Bp dynamics after challenge in nasopharyngeal

		swabs (culture, qPCR and microbiome analyses), nasal wash (culture including semi-quantitative method using cfu count/mL, and precision quantification with qPCR) and sequencing of isolates at various time-points
6	To assess environmental shedding of Bp following nasal inoculation of healthy participants with Bp.	Daily microbiological assays from day 0 to 16 to detect Bp on surface contact (culture and PCR), air sampling (PCR) fingertip culture (culture Bp and PCR), cough box (culture Bp, particle size during various activities: talking, coughing, singing)
7	To determine the eradication rate of Bp after a three day course of Azithromycin	Microbiological assays after eradication in nasopharyngeal swabs (culture, qPCR and microbiome analyses), nasal wash (culture including semi-quantitative method using cfu count/mL, qPCR) on day 15 and 16
8	To describe the human immune response to challenge, including innate, humoral, cell-mediated and mucosal responses.	Immunological assays to measure innate, humoral, cell-mediated and mucosal responses to challenge in blood (anti-PT IgG (IU/mL), anti-FHA IgG (IU/mL), anti-PRN IgG (IU/mL), anti-FIM IgG (IU/mL), nasal washes (T-cell/B-cell analyses), nasal fluid samples (cytokines) and saliva (Bp specific IgA (IU/mL)) samples, comparing day 0 with day 1, 3, 4, 7, 9, 11, 14, and week 4, 8, 26 and 52.

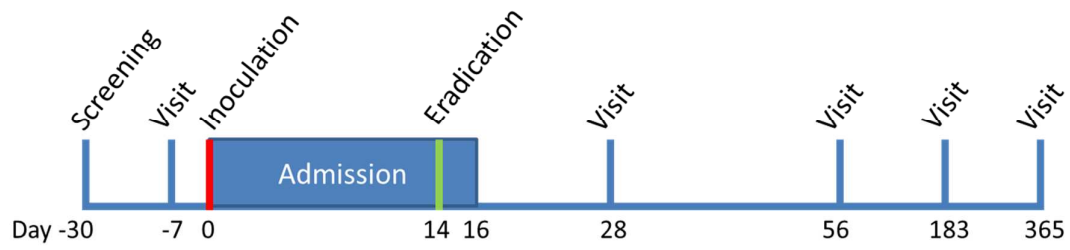
Bp: *B. pertussis*, cfu: colony-forming units, CRP: C-reactive protein, WBC: white blood cell count, PT: pertussis toxin, PCR: polymerase chain reaction, qPCR: quantitative PCR, IU: international units, PRN: pertactin, FHA: filamentous hemagglutinin, FIM: fimbriae

Table 2: Scheduled events and interventions:

Day	-30	-7	0	1	2	3	4	5	7	9	11	14	15	16	28	56	183	365
Visit	x	x													x	x	x	x
Admission																		
Inoculum			x															
Bloods	x		x	x	x	x	x		x	x	x	x			x	x	x	x
Urine	x		x															
ECG	x																	
Nasal wash		x					x		x	x	x	x		x	x	x	x	x
Nasal fluid sample		2x				2x	2x	2x	2x	2x	2x	2x	2x	2x	2x	2x	2x	2x
Nasopharyngeal swab	x	x				x		x	x	x	x	x	x	x	x			
Throat swab		x				x		x	x	x	x	x	x	x				
Saliva		x				x		x	x	x	x	x	x	x				
Antibiotic therapy												x	x	x				

ECG: Electrocardiogram

Figure 1: Visits and admission design



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Figure 2: Escalating the dose of the inoculum according to colonisation rate

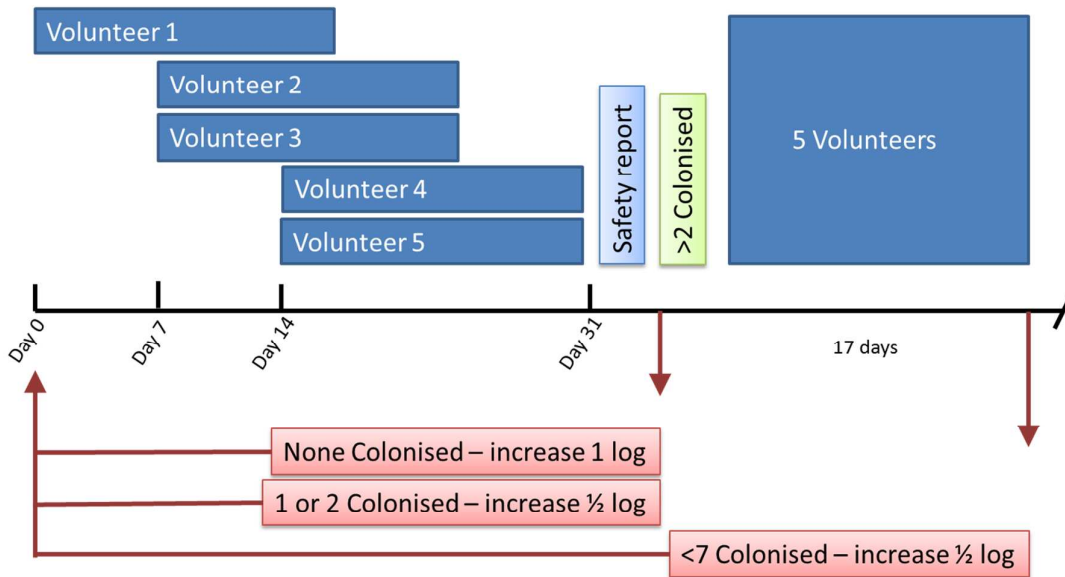
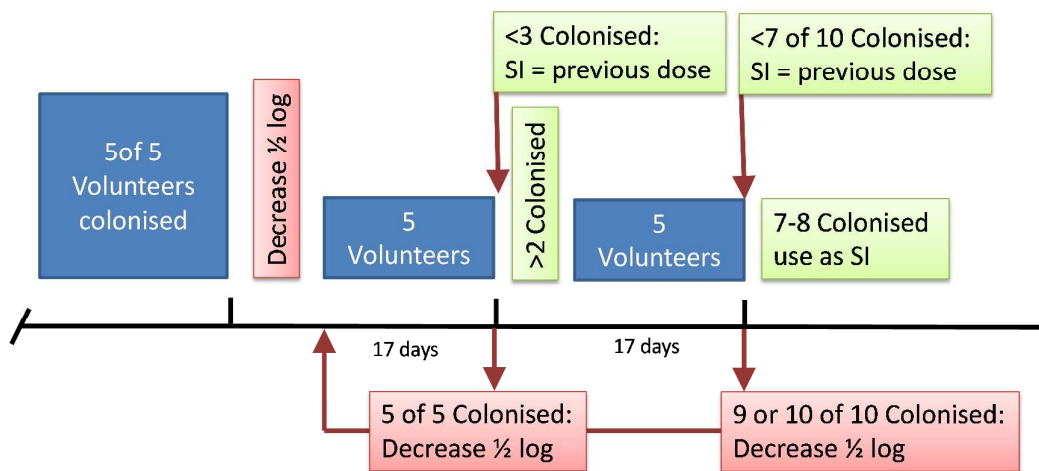


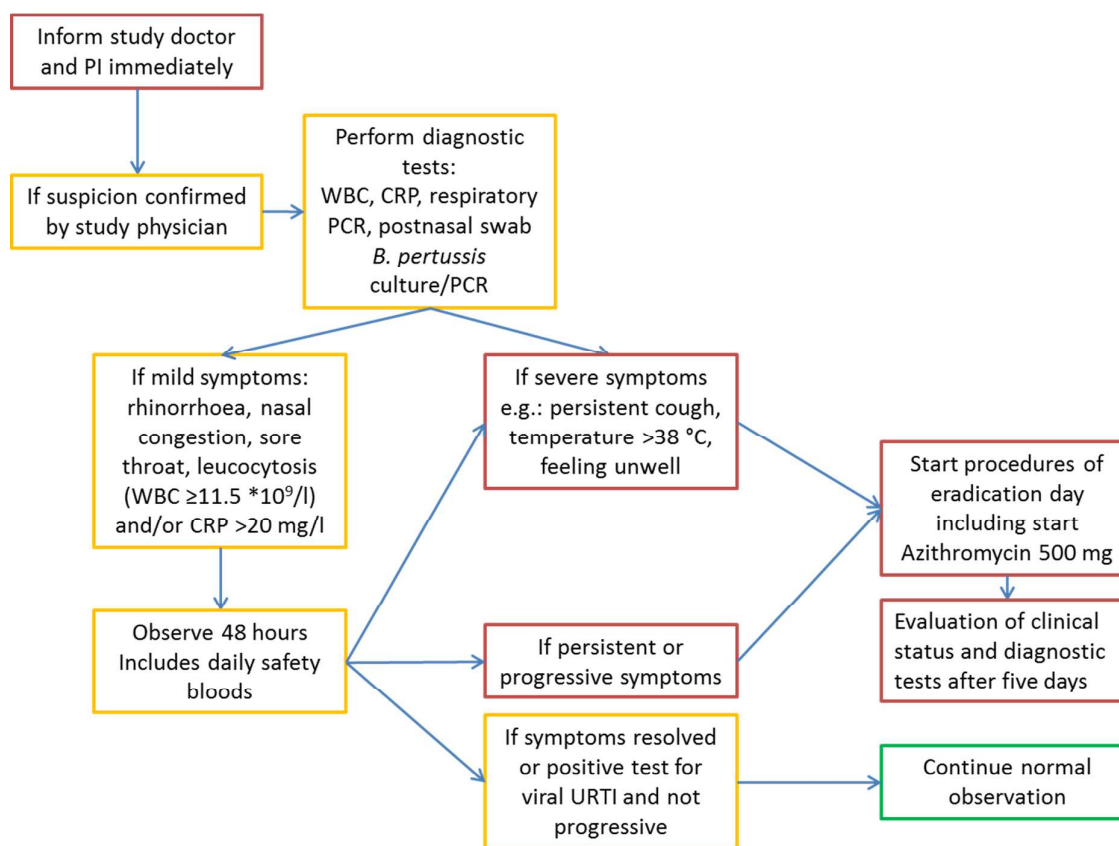
Figure 3: De-escalating the dose of the inoculum according to colonisation



SI: standard inoculum dose

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Figure 4: Actions to be taken when symptoms of early *B. pertussis* disease are suspected

PI: Principal investigator, WBC: White blood cell count, CRP: C-reactive protein, PCR: polymerase chain reaction, URTI: Upper respiratory tract infection

Supplement table 1:

Inclusion and exclusion criteria for the *B. pertussis* human challenge colonisation study

Inclusion criteria	
1	Healthy adults aged 18 to 45 years inclusive at screening
2	Fully conversant in the English language
3	Able to communicate easily by both mobile telephone and text messaging
4	Able and willing (in the investigator's opinion) to comply with all study requirements
5	Written informed consent to participate in the trial
6	Willingness to take an antibiotic regimen after inoculation with <i>B. pertussis</i> according to the study protocol
7	Agreement to be admitted to the NIHR-CRF Southampton for 17 days for phase A (from inoculation until two days after the eradication therapy is given) and for the duration necessary for phase B, depending on phase A results
8	Agree to the infection prevention rules as stated in the volunteer information sheet
9	TOPS registration completed and no conflict found

NIHR-CRF: National Health Institute for Health Research-Clinical Research Facility, TOPS: The Over-volunteering Prevention System

Exclusion criteria	
1.	Individuals who have inviolable commitments within 3 months of discharge from the inpatient phase of the study to make contact with: a. unimmunised or partially immunised children and infants aged < 1 year b. pregnant women >32 weeks who have not received pertussis vaccination at least a week before contact
2.	Individuals who have household contacts working with a. unimmunised or partially immunised children and infants aged < 1 year b. pregnant women >32 weeks who have not received pertussis vaccination at least a week before contact
3.	Phase A only: Volunteers will be excluded from this study if they have evidence of recent <i>B. pertussis</i> infection, as determined by anti-PT IgG ELISA (>20 IU/mL)
4.	<i>B. pertussis</i> detected on nasopharyngeal swab taken before the challenge
5.	Individuals who have a signs of a current infection at the time of inoculation with <i>B. pertussis</i>
6.	Individuals who have participated in other interventional clinical trials in the last 12 weeks
7.	Individuals who have a history of receiving <i>B. pertussis</i> vaccination in the last 5 years
8.	Individuals who have a history of never being vaccinated against <i>B. pertussis</i>
9.	Current smokers defined as having had a cigarette/cigar in the last week.
10.	Use of systemic antibiotics within 30 days of or during the challenge
11.	Any confirmed or suspected immunosuppressive or immune-deficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) immunosuppressant medication within the past 6 months (topical steroids are allowed)
12.	Use of immunoglobulins or blood products within 3 months prior to enrolment
13.	History of allergic disease or reactions likely to be exacerbated by any component of the inoculum
14.	Contraindications to the use of azithromycin or macrolides
15.	Pregnancy, lactation or intention to become pregnant during the study

16.	Any clinically significant abnormal finding on biochemistry, haematology, toxicology or serological blood tests, urinalysis or clinical examination - in the event of abnormal test results, confirmatory repeat tests will be requested
17.	Any other significant disease, disorder, or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data, for example recent surgery to the nasopharynx

PT: pertussis toxin, IgG: immunoglobulin-G, ELISA: enzyme-linked immunosorbent assay, IU: international units

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3 *Effective contraception for female participants*
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5 Female participants are required to use an effective form of contraception during this study.
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7 Acceptable forms of contraception include:
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10 - Established use of oral, injected or implanted hormonal methods of contraception
11 - Placement of an intrauterine device or intrauterine system
12 - Total abdominal hysterectomy
13 - Barrier methods of contraception (condom or occlusive cap with spermicide)
14 - Male sterilisation if the vasectomised partner is the sole partner for the subject
15 - True abstinence when this is in line with the preferred and usual lifestyle of the subject
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The logo for 'peri scope' features the word 'peri' in a teal sans-serif font above the word 'scope' in a darker teal sans-serif font. The letter 'o' in 'scope' is replaced by a circular graphic containing several red dots, resembling a microscope's field of view or a cluster of cells.

**A human controlled infection study to establish a safe, reproducible and practical human *Bordetella pertussis* colonisation model for the identification of correlates of protection against colonisation.
(BPCCS)**

Sponsor Study reference: TBC

REC Study Reference: 17/SC/0006

IRAS ID: 219496

Protocol version: 2.0

Date: 15/02/2017

Chief investigator: Professor R.C. Read

University Hospital Southampton NHS Foundation Trust

Version	Date	Author(s)
1.0	7 nd Nov. 2016	Hans de Graaf, Robert Read
1.1	19 th Nov 2016	Hans de Graaf, Robert Read
2.0	15 th Feb 2017	Hans de Graaf, Robert Read

Amendments	
1.1	Paragraph.16.2 Insurance wording changed
2.0	Daniela Fereira removed from the External Safety committee. Adjustments following REC and HRA review

1
2
3 **A human controlled infection study to establish a safe, reproducible and**
4 **practical human *Bordetella pertussis* colonisation model for the identification**
5 **of correlates of protection against colonisation**
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Confidentiality Statement

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

Investigator Agreement

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

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

Professor R.C. Read
Chief Investigator	Investigator Signature	Date

Conflict of Interest

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

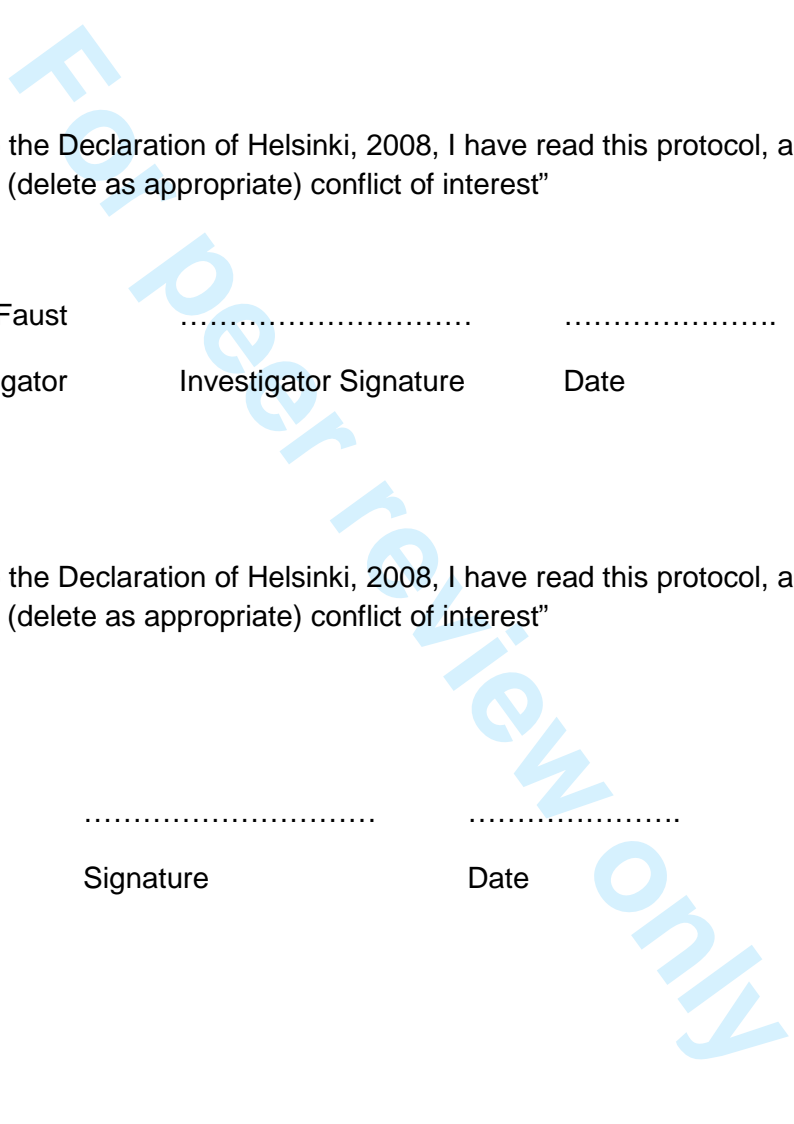
Professor R.C. Read
Chief Investigator Investigator Signature Date

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

Professor S.N. Faust
Principal Investigator Investigator Signature Date

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

H. de Graaf
Investigator Signature Date



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Synopsis

Title	A human controlled infection study to establish a safe, reproducible and practical human <i>Bordetella pertussis</i> colonisation model for the identification of correlates of protection against colonisation
Sponsor	University of Southampton - TBC
Trial Centre	NIHR Wellcome Trust Clinical Research Facility, Southampton University Hospital NHS Foundation Trust, Southampton, SO16 6YD
Trial Sponsor Code	TBC
Design	Single centre controlled human <i>Bordetella pertussis</i> colonisation study. Intervention group: nasal challenge with <i>Bordetella pertussis</i> . Control group: nasal challenge with sterile saline.
Type of study	First in human study
Population	Healthy volunteers aged 18-45 years
Sample size	80 healthy volunteers in total Phase A: Determining the standard inoculum. Pilot <i>B. pertussis</i> human challenge model development – 35 volunteers Phase B: Development of a <i>B. pertussis</i> human challenge model <ul style="list-style-type: none"> - Intervention group - 30 volunteers - Control group - 15 volunteers
Follow up duration	Phase A - Challenge on day 0, eradication on day 14: admission for 17 days, follow up on day 28, 56, 182, and 364. Phase B - Challenge on day 0, eradication on day 7: <ul style="list-style-type: none"> - Intervention group: Day of eradication and length of admission will depend on results of phase A. Follow up day at day 14, 28, 56, 182, and 364. - Control group: No admission, follow up day at day 28, 56, 182, and 364.
Planned Trial Period	01/03/2017 – 31/12/2021

Primary Objective	To develop a safe controlled human challenge colonisation model with <i>B. pertussis</i>
Secondary Objective	<ul style="list-style-type: none">- To establish 70% asymptomatic colonisation of <i>B. pertussis</i> in healthy volunteers after nasal inoculation with <i>B. pertussis</i>.- To assess <i>B. pertussis</i>-specific immunity in healthy volunteers before and after nasal inoculation with <i>B. pertussis</i>- To assess environmental shedding of <i>B. pertussis</i> following nasal inoculation of healthy volunteers with <i>B. pertussis</i>.
Microbial challenge material	<i>B. pertussis</i> strain B1917, dose between 10^2 and 10^6 cfu.

Abbreviations

AE	Adverse Event
ALS	Advanced life support
BMGF	Bill and Melinda Gates Foundation
CFU	Colony Forming Unit
aP	Acellular pertussis vaccine
Bp	<i>Bordetella pertussis</i>
CRF	Case Report File
CRP	C-Reactive Protein
DMSC	Data Monitoring and Safety Committee
DSMB	Data and Safety Management Board
DSUR	Development Safety Update Report
EAG	Ethical Advisory Group
EFPIA	European Federation of Pharmaceutical Industries and Associations
ESC	External Safety Committee
FIH	First in Human
GCP	Good Clinical Practice
GHQ	General Health Questionnaire
GP	General Practitioner
HB	Haemoglobin
HPA	Health Protection Agency
HRA	Health Research Authority
ICH	International Conference on Harmonisation
ICU	Intensive Care Unit
ILS	Immediate Life Support
IMI	Innovative Medicines Initiative
IQR	Interquartile range

MHRA	Medicines and Healthcare Products Regulatory Agency
NHS	National Health Service
NIHR-WTCRF	National Institute for Health Research Wellcome Trust Clinical Research Facility
NIP	National Immunisation Program
PBS	Phosphate Buffered Saline
PHE	Public Health England
PPE	Personal Protective Equipment
PCR	Polymerase Chain Reaction
PI	Principal Investigator
PT	Pertussis Toxin
QA	Quality Assurance
QC	Quality Control
REC	Research Ethics Committee
SAE	Serious Adverse Event
SMPC	Summaries of Product Characteristics
SAB	Scientific Advisory Board
SOP	Standard Operating Procedures
SUSAR	Suspected Unexpected Serious Adverse Reaction
TOPS	The Over-volunteering Prevention System
UAR	Unexpected Adverse Reaction
UHS NHS FT	University Hospital Southampton NHS Foundation Trust
UK	United Kingdom
VIS	Volunteer Information Sheet
WBC	White Blood cell Count
WHO	World Health Organisation
wP	Whole-cell pertussis vaccine

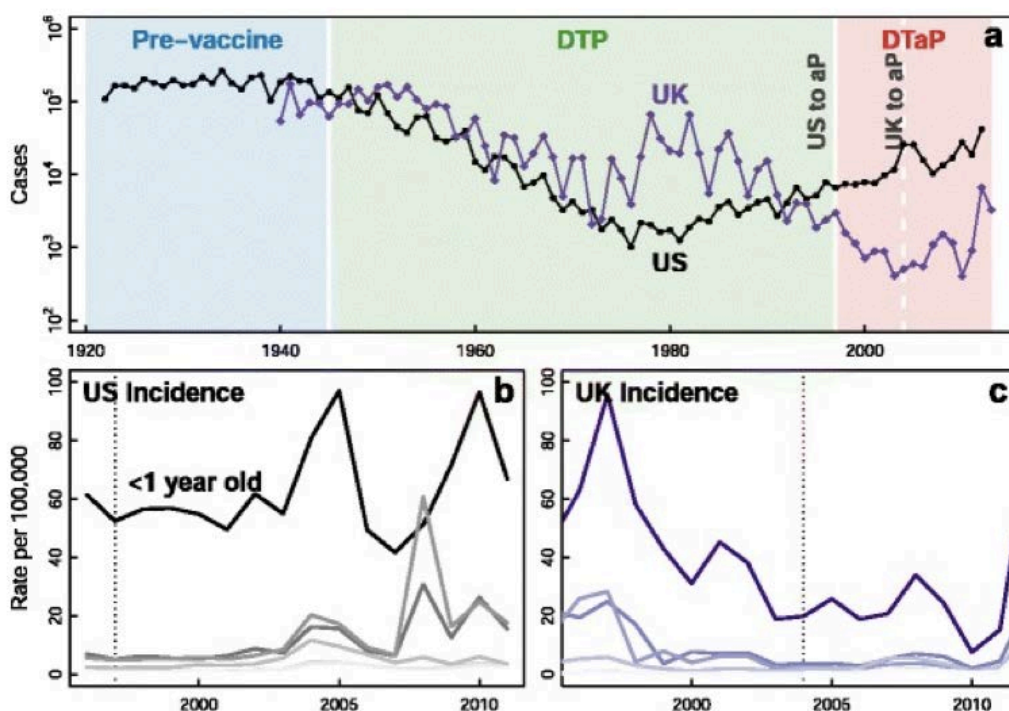
1. Background and rationale

1.1 Pertussis

Pertussis, also called whooping cough, is an acute bacterial infection caused by *Bordetella pertussis* (Bp), an exclusively human pathogen. Although it can affect people of all ages, young unimmunised infants are the most vulnerable group with the highest rates of complications and death (1).

The highest morbidity and mortality due to pertussis remain in low-income countries, where vaccine coverage is often still low. In 1999 there were an estimated 48.5 million pertussis cases in children worldwide and 295,000 deaths (2). Between 2003 - 2013 mortality fell to about 60,600 (interquartile range (IQR) 22,300-136,800) children per year, still making it one of the leading causes of vaccine-preventable death (3). A European surveillance between 1994 and 1998 showed that 0-5% of the English and Welsh population was positive (>62.5 IU/mL) for anti-PT IgG, which was relatively low in comparison to the other European countries (4). But there appears to be a significant difference in incidence rates obtained from the seroprevalence studies and from the national reporting systems (5). The latest reported incidence of pertussis in the UK in 2015 was 8.1/100000 (6). Many countries have seen an increase in the incidence of pertussis over the past 20 years (7) (see Figure 1). Four main hypotheses have been proposed to contribute to this resurgence: 1) waning of protective immunity from vaccination or natural infection over time, 2) evolution of *B. pertussis* to escape protective immunity, 3) low vaccine coverage and 4) asymptomatic transmission from individuals vaccinated with the currently used acellular *B. pertussis* vaccines (aP) (8).

Figure 1: Increase in *B. pertussis* incidence over time.



Panel a shows *B. pertussis* cases in the United States from 1922 through 2012 and in the United Kingdom from 1940 through 2013. Shaded regions correspond to the pre-vaccine era, the DTP era, and the DTaP era, respectively. Dotted white line: introduction of acellular pertussis vaccine in routine immunisation program UK.

1
2
3 Panels b and c show the incidence of *B. pertussis* by age group with darker color indicating younger ages in the
4 US and UK, respectively. Infants less than 1 year old are labelled in darkest colours. Figure adapted from (8)
5

6 **Asymptomatic colonisation**

7
8 A recent mathematical study using both epidemiological and genomic data gives strong
9 empirical support for asymptomatic transmission and suggests that the use of the aP
10 vaccine, which blocks symptomatic disease but not asymptomatic transmission, may
11 account for the observed increase in *B. pertussis* incidence (Althouse, BMC Med. 2015; 13:
12 146). Direct evidence for asymptomatic colonisation has proven hard to obtain. In a study in
13 1968 nasopharyngeal swabs were taken from 1102 individuals, including groups of healthy
14 babies, preschool and school children, and family and neighbourhood contacts of cases,
15 during a year when pertussis was epidemic. No asymptomatic carriers were demonstrated. It
16 was suggested that *B. pertussis* is carried in a non-culturable form which would prevent
17 detection by the methods used (9).
18

19
20
21 In a more recent Chinese study nasopharyngeal swabs were taken from 629 asymptomatic
22 school children aged 7 to 15 years. 2 (0.3%) were culture positive and 30 (4.8%) were PCR-
23 positive for *B. pertussis*. (10).
24

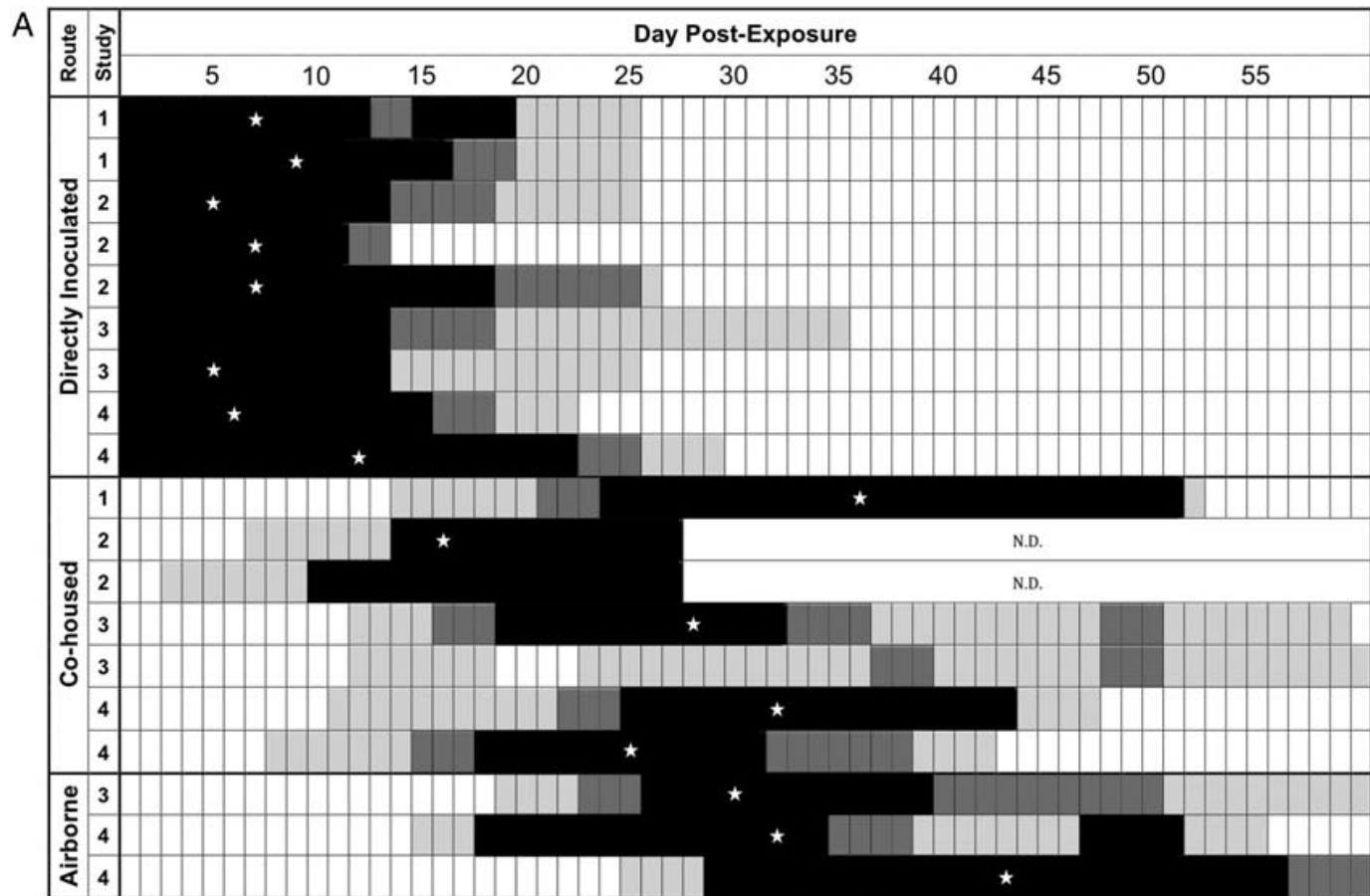
25
26 A further study looking at colonisation of *B. pertussis* in college students in Virginia, USA, is
27 currently ongoing (11).
28

29
30 Positive anti-PT serology without evidence of recent vaccination or infection could indicate
31 asymptomatic colonisation in adults. A Finnish household study from 1983 reported to find
32 46% of the secondary cases to be asymptomatic (12). A more recent Dutch study looking at
33 household members of children admitted because of pertussis showed 17.6% of the adults
34 had a serological diagnosis without symptoms (13). Unfortunately no recent serology data is
35 available from the UK.
36

37 **Transmission**

38
39
40 Transmission of the organism occurs via airborne droplets as a result of close contact with
41 an infected person. In a study looking at children hospitalised with pertussis, *B. pertussis*
42 DNA was detected in air samples taken as far away as 4 metres (13 feet) from a patient's
43 bedside for up to 4 days following initiation of therapy although the clinical relevance of this
44 remains uncertain (14). In a recent study weanling baboons were either inoculated directly
45 with *B. pertussis* (via intranasal and endotracheal route) or co-housed in the same cage as
46 these inoculated baboons, or housed in a caging unit 7 feet away from the inoculated
47 baboons. It demonstrated the time to infection of the distally housed animals was
48 significantly longer than the time to infection of the cohoused animals (19 vs 10 days,
49 respectively; $P = .0027$) (15) (See Figure 2)
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Figure 2: Colonisation and leucocytosis in directly inoculated and exposed baboons (15).



White = 0, light gray = 1–200 colonies, dark gray = 201–2000 colonies, and black = >2000 colonies in nasal wash. The stars indicate the day of peak leucocytosis for each animal except for the 2 animals that did not achieve a peak at least 2-fold higher than baseline. N.D.: Not determined.

Epidemiology studies show that *B. pertussis* is highly contagious, each case infecting between 5.5 and 22 others, with up to 90% of household contacts developing the disease (16). Individuals with pertussis usually spread the disease to another person by coughing or sneezing or by sharing breathing space for a prolonged time.

Incubation period

The initial contact with *B. pertussis* is usually hard to determine in cohort studies and little is known about colonisation of *B. pertussis* before it causes symptoms. The incubation period is dependent on dose, which is related to the intensity and length of exposure, and the immune status of the host. This immune response is related to age and previous exposure to pertussis antigens (vaccination, colonisation or infection).

In an experimental challenge study done in 1933, four children aged six to nine years were inoculated via the upper respiratory tract with approximately 140 bacilli (17). None of them had a history of whooping cough and two of them were vaccinated 5 months prior to the challenge. The two boys who had not been previously vaccinated began to cough on day 7. By that time they had a positive cough plate and had normal blood counts. Within a week their coughing had become increasingly severe and they developed a leucocytosis of 17,250 and 21,500, with 40-50% lymphocytes. By day 17 the paroxysms became more prolonged

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3 and intense, and they developed fever. The following week the typical whoop was heard and
4 cough plates were negative. At day 28 there was daily whooping, with vomiting of mucus
5 and food, headache after severe paroxysms, anorexia and the leucocytosis reached a
6 maximum of 29,200 and 22,600 with 38-56% lymphocytes. By day 35 the symptoms became
7 less severe and the boys recovered. This study suggested an incubation period of seven
8 days in these two children who were never exposed to *B. pertussis* and were inoculated
9 experimentally.
10
11

12
13 The baboon model (15) showed that baboons inoculated via the endotracheal and intranasal
14 routes with a high dose of pertussis (10^9 CFUs) are colonised on day 1, and shed sufficient
15 numbers of organisms to cause colonisation in co-housed baboons at day 3 (median 11,
16 range 3-14 days). Colonisation due to airborne transmission (in distally housed animals) was
17 caused on day 15, 19 and 25. Colonisation is seen before symptoms occur. Following the
18 first positive bacterial culture, the infection builds over a period of 1-3 weeks. The frequency
19 of cough and the severity of leucocytosis seems to be related to the level of CFUs in the
20 nasal washes (personal correspondence T. Merkel).
21
22

23
24 A Dutch study reported that the median serial interval for *B. pertussis*, defined as the interval
25 between symptom onset of a secondary case and that of its primary case, is 22 days (95%
26 confidence interval 10-35 days) (18). The weakness of the serial interval is that it is not
27 certain at which point transmission occurs. **Therefore the accurate incubation period**
28 **remains unknown, but from the information above, probably falls in the range 7-22**
29 **days.**
30
31

32 **Clinical features**

33
34 Symptoms of pertussis in infected children typically occur in three different stages. The first
35 stage is the catarrhal stage, which lasts for 1-2 weeks, and is characterised by non-specific
36 symptoms such as rhinorrhoea, sneezing, low-grade fever and cough. The second stage is
37 the paroxysmal stage, lasting for 1-6 weeks, and is characterised by pathognomonic
38 symptoms of pertussis such as episodes of paroxysmal cough with a characteristic
39 whooping sound. The paroxysmal cough can lead to post-tussive cyanosis and emesis. The
40 final stage is the convalescent stage in which the respiratory symptoms gradually decrease
41 although coughing may last for several months (19). Patients with pertussis are most
42 infectious in the initial catarrhal stage and during the first three weeks after the onset of
43 cough.
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48 As mentioned above, serological studies looking at anti-Pertussis toxin (PT) IgG titres in
49 healthy adults do suggest that *B. pertussis* infections in adults are often unrecognised or
50 asymptomatic (12, 20, 21). During a 5 year follow up 90% of health care workers had
51 evidence of one pertussis toxin antibody rise without symptoms, 55% had two episodes,
52 17% had three episodes and 4% had 4 episodes (22).
53
54

55 If pertussis is diagnosed in **adults**, reported symptoms of *B. pertussis* disease are mild in
56 general, often atypical and include: coughing 91% (mean duration: 54 days), in 80% this
57 cough lasts ≥ 21 days. Other symptoms include whoops (8%), cough followed by vomiting
58 (53%) and cough disturbing sleep (52%), sweating attacks (14%), pharyngeal symptoms
59 (37%), influenza-like symptoms (30%), sneezing attacks (22%), hoarseness (18%), sinus
60 pain (16%) and headaches (14%) (23).

Complications

The most commonly reported complications of pertussis in adults include insomnia, apnoea, weight loss, urinary incontinence, syncope, and rib fractures, all due to cough (see Table 1) (26). Less common complications include pneumonia, otitis media, and, very rarely, death (24, 25). In the U.S, five pertussis-associated deaths in adults were reported to the CDC between 1990 and 2004 (26). All of them were older than 48 years, and suffered from comorbid conditions.

Table 1: Complications of pertussis in adolescents and adults, all ages reported by Kilgore et al. (27)

	Frequency (%)
Apnoea	27–86
Pneumonia	0.6–8
Convulsions	0–0.6
Death	0.01
Insomnia	77
Sinusitis	13
Otitis media	4
Weight loss	3–33
Urinary incontinence	3–28
Syncope	2–6
Rib fracture	1–4
Loss of consciousness	1
Hospitalisation	0–12

Treatment

Antibiotic treatment is shown to be effective in eradicating *B. pertussis*, but does not alter the subsequent clinical course of the illness (28), especially if administered beyond 2–3 weeks **after** the onset of symptoms. Azithromycin has been shown to be effective at rapidly eradicating *B. pertussis* from the nasopharynx with clearance of 97% of individuals at day two to three after starting treatment (29). The use of antimicrobial therapy late in the course of disease is less likely to affect pertussis symptoms than use early in the course of disease (30). Azithromycin is known to have an immunomodulatory effect on innate and adaptive immune responses. The drug appears to exert a biphasic action which may serve to promote initial host defence and later reduce bystander tissue injury and promote inflammation resolution (31).

Vaccination provides the most effective strategy for preventing pertussis, although protection afforded by vaccination or from past infection is not lifelong.

The need for improved vaccines

Pertussis vaccines have been one of the cornerstones of National Immunisation Programmes (NIP) since their introduction in the 1940s-1950s. The widespread use of whole-cell pertussis vaccines (wP) in NIPs resulted in a huge reduction of pertussis-related

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3 deaths and disease, especially in young infants. Currently, acellular pertussis vaccines (aP),
4 which have a more favourable reactogenicity profile, are effectively used in industrialised
5 countries to immunise against pertussis.
6

7
8 However, there are concerns about the ability of aPs to provide a similar durability of
9 protection as that induced by wPs. Over the last two decades, evidence has accumulated
10 showing that the true burden of disease caused by *B. pertussis* in industrialised countries is
11 much larger than previously assumed. There has been a resurgence of disease, particularly
12 in vaccinated populations such as older children and the elderly, which are not typically
13 considered to be at risk (see Figure 1). Of concern, in several countries an increase has
14 been observed in the circulation of *B. pertussis* strains that do not express one or more
15 vaccine antigens (32). Epidemiological, clinical and preclinical studies have shown that
16 immunity in humans wanes rapidly after immunisation with pertussis vaccines, especially
17 with aP (33). This suggests that the improvements in the reactogenicity profile of aP, as
18 compared to wP, may be accompanied by differences in the elicited immune response.
19 Studies in the baboon model have demonstrated that aPs prevent severe disease but do not
20 prevent asymptomatic infection, ie colonisation (34). Studies in mice are consistent with
21 these findings and demonstrate that protective immunity is more effective and persistent
22 when induced by infection or wP than by aP. Infection and immunisation with wP both
23 induce potent induction of T cell immunity, in particular IFN- γ -secreting CD4 T cells (Th1
24 cells), whereas aP vaccines induce strong antibody responses and Th2-type responses (35).
25 There is also evidence of a role for Th17 cells, although this has not yet been confirmed in
26 humans. The role of functional antibodies and T cell responses in protection against disease
27 and/or colonisation has been demonstrated previously by different laboratories (36). To
28 study the pathogenesis of pertussis a variety of animal models have been used, including
29 mice, rabbits, guinea pigs, and newborn piglets (37). However, there are still important
30 knowledge gaps relating to human immunity to *B. pertussis* and it is not clear to what extent
31 these observations in animal models translate into clinical practice.
32
33

34
35 This paucity of knowledge hampers the development of improved vaccines and the design of
36 better vaccination strategies against pertussis in infants, adolescents and adults. The
37 development and licensing of the next generation of vaccines depends on 1) A more
38 thorough understanding of the immune response to infection 2) The identification of
39 biomarkers of protective immunity and 3) The development of human and animal models as
40 well as bioassays to predict and evaluate vaccine efficacy. The development of a safe
41 human challenge model of pertussis, in conjunction with the recently developed baboon
42 model of pertussis, has the potential to provide a path forward for answering critical
43 questions about pertussis pathogenesis and host responses and will likely aid in the
44 development of next-generation pertussis vaccines (38).
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52 1.2 The *B. pertussis* human challenge model

53 *Potential advantages of a human challenge mode*

54
55 The initial colonisation of host surfaces is poorly understood but is a key step required for the
56 spread and pathogenesis of *B. pertussis*. A human challenge model to establish colonisation
57 would provide an opportunity to study the initial colonisation dynamics and host response,
58 including possible immune correlates of protection. It would allow analysis of time taken to
59 establish colonisation and as well as biomarkers of local and systemic immune response
60 triggered by exposure to *B. pertussis*. A safe *B. pertussis* challenge model will also provide

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3 the ability to test candidate vaccines and compare immune responses caused by
4 experimental challenge with that caused by vaccination.
5

6
7 For ethical reasons, because no effective treatment option to treat disease is available at the
8 moment, the human challenge model can only be used to measure only colonisation.
9 Therefore, any markers found to be associated with protection from colonisation in the
10 human challenge model will have to be cross-validated in the baboon challenge model and
11 in a family transmission setting in the Periscope consortium.
12

13 *Previous experience with nasal microbial challenge models*

14
15 The first human challenge with *B. pertussis* was performed in 1933, exposing four children,
16 of which two might have been naive to *B. pertussis* (see above). In a recent first in human
17 study healthy volunteers were given a live attenuated *B. pertussis* strain as a nasal vaccine
18 (39). The inoculated strain was genetically modified; dermonecrotic toxin and tracheal
19 cytotoxin were removed, and PT was genetically detoxified by two independent mutations,
20 affecting the toxic activity of PT without affecting the immunogenic properties. The effect of
21 the genetic changes on the ability to colonise are unknown. Three groups, each of 12
22 volunteers, were inoculated with 10^3 , 10^5 or 10^7 colony forming units respectively.
23 Colonisation was seen in one subject in the low dose, one in the medium dose and five in
24 the high dose group. Significant increases in immune responses against all pertussis
25 antigens were seen in all colonised subjects. Adverse events occurred in similar frequency in
26 all groups, including the placebo group and were found to be trivial. Because of the genetic
27 changes of the attenuated *B. pertussis* used in this study, the dose of the inoculum and
28 colonisation dynamics might differ from wild type *B. pertussis* colonisation.
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34 In a previous study we developed an experimental human challenge model of *Neisseria*
35 *lactamica* colonisation to investigate the role of *N. lactamica* in natural immunity to the
36 meningococcus (40). In the study we enrolled 61 volunteers with no current carriage of
37 *Neisseria* spp. and inoculated 41 of them intra-nasally with 10,000 colony forming units of *N.*
38 *lactamica* and 20 with phosphate-buffered saline (PBS) control. Colonisation was monitored
39 in oropharyngeal samples over 6 months by conventional microbiological techniques and
40 confirmation using PCR specific for the inoculated strain. Of the 41 who were challenged
41 intra-nasally with 10^4 cfu of *N. lactamica* 26 (63.4% [95% CI 49.5- 77.9%]) became
42 colonised. In this group, carriage of *N. lactamica* remained stable over 12 weeks, and 17
43 remained colonised at 24 weeks. In all cases of *N. lactamica* carriage, the isolate was typed
44 as the inoculum strain by PCR. All 15 individuals who were challenged with, but not
45 colonised by *N. lactamica* remained culture negative for *N. lactamica*. Following a second
46 challenge with 10^4 cfu *N. lactamica*, 2/11 volunteers were colonised, a reduction in carriage
47 rate from 100% to 18%. Likewise 13 of the 15 who were originally challenged but not
48 colonised were re-challenged with *N. lactamica* but none became colonised. Of these, 6
49 were then re-challenged with a higher dose of 10^5 cfu and 3 (50%) became colonised. In the
50 study an additional cohort was inoculated with *N. lactamica* shortly after parenteral
51 vaccination with *N. lactamica* outer membrane vesicles (OMV) produced from the same
52 strain. OMV vaccinees had high levels of systemic and mucosal anti-*N. lactamica* antibodies
53 and were relatively resistant to *N. lactamica* carriage.
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60 In a second *N. lactamica* nasal challenge study 310 university students were inoculated with
 10^4 colony-forming units of *N. lactamica* or were sham-inoculated, and carriage was
monitored for 26 weeks, after which all participants were reinoculated with *N. lactamica* and

resampled 2 weeks later. It showed that the inhibition of meningococcal carriage by *N. lactamica* is even more potent than after glycoconjugate meningococcal vaccination (41).

Prof R. Read and Dr D. Diavotopoulos have been involved in the development of the pneumococcal human challenge model in Liverpool. In this model healthy volunteers were nasally inoculated with increasing doses of *S. pneumoniae* and carriage and immune responses were analysed (see Figure 3). In total 29 out of 70 subjects were experimentally colonised. Carriage increased both mucosal and serum IgG levels to pneumococcal proteins and polysaccharide, resulting in a fourfold increase in opsonophagocytic activity. No subjects were colonized by experimental re-challenge, demonstrating the protective effect of initial carriage against subsequent infection. The doses required for colonisation (10^4 - 10^5) were much lower than those used in animal models (10^7) (42).

Figure 3: Dose-response curve of experimental carriage in *S. pneumoniae* challenge model

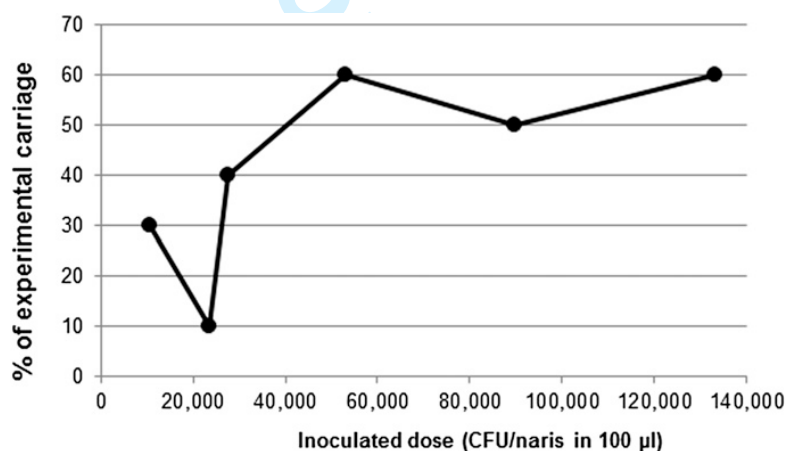


Figure 2. Groups of 10 volunteers were inoculated with 100 ml of increasing doses of 6B Streptococcus pneumoniae (10,000, 20,000, 40,000, 80,000, and 160,000 cfu/naris). An extra cohort (n = 20) was inoculated with 40,000 cfu/naris. Inoculated doses were quantified by Miles and Misra dilution and plating. The average of inoculated doses and corresponding percentage of carriage achieved for each group are indicated.

The *B. pertussis* challenge model to establish colonisation can be done safely

Our priority is to develop this model without causing harm to the individual or the environment. An important facet of a safe model will be to limit participant exposure to *B. pertussis* colonisation to the minimum number of days required to signal successful colonisation.

1. The dose of the inoculum will start low and will be modified to the lowest dose required to effectively establish colonisation with regular reviews of the available safety data by the study steering committee and the external safety committee (see paragraph 4.4).
2. The timing of eradication will be modified to optimise the safety of the volunteers by exposing them to the minimal effective period of colonisation, with frequent reviews of available safety data by the study steering committee and the external safety committee (see paragraph 4.4).
3. After inoculation of the volunteers, there will be a moderate risk of symptom development because eradication therapy will initially be given at day 14, while the incubation time for the catarrhal phase is unknown but is estimated to be 7 – 22 days (see paragraph 1.1).

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4. If a volunteer shows signs of possible disease there will be a low threshold to eradicate colonisation and treat possible infection (see paragraph 8.5).
 5. Disease in adults is often atypical and relatively mild. Treatment is not thought to reduce the duration of coughing in natural infection, but adults with natural infection will not usually receive treatment until a late stage of the disease, if at all. Early treatment (as proposed in this study) is considered more likely to reduce symptoms quickly (see paragraph 1.1).
 6. The likelihood of severe disease is extremely low and the risk of harm to the volunteers, staff and others can be minimised by admitting the volunteers as inpatients in the Southampton NIHR-WTCRF which is a NIHR facility specifically funded to conduct higher risk experimental medicine in a safe NHS environment.

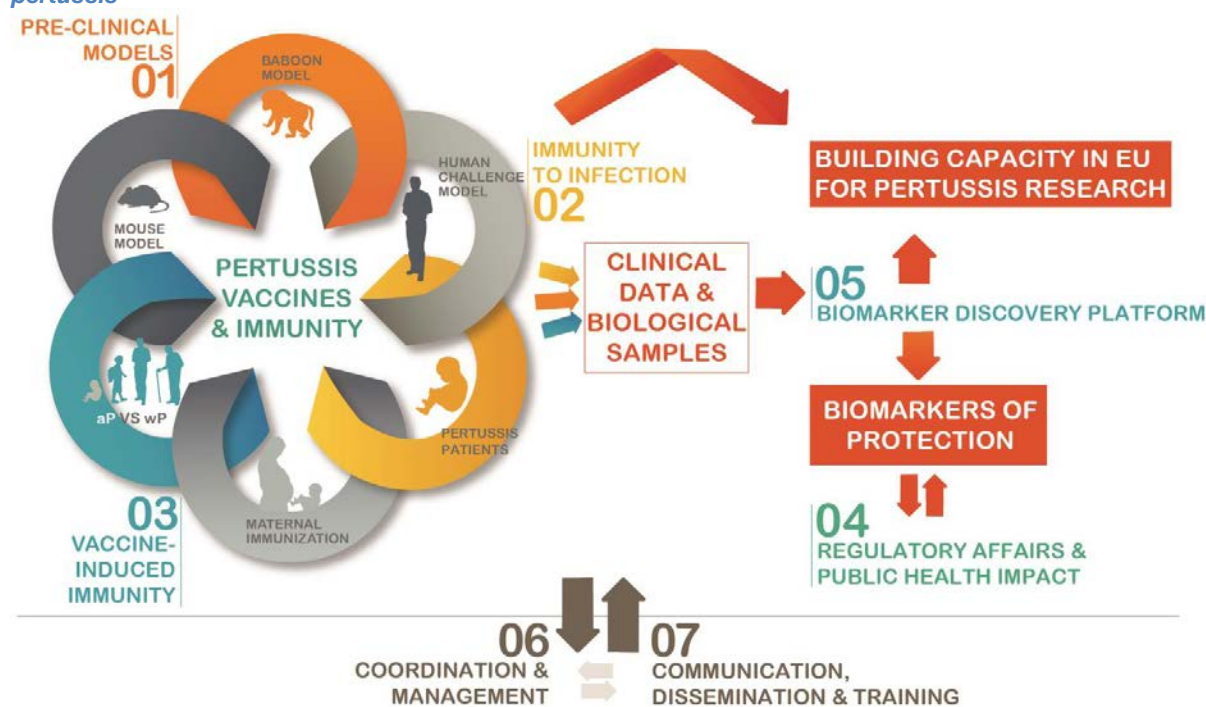
A phased approach will be used to reduce the time of isolation and possibly to adapt the model, if deemed safe, from an inpatient to an outpatient setting. The NIHR-WTCRF in Southampton and the involved partners have the required expertise and the facilities to perform this study in a safe and controlled manner.

Once adequate information is provided, it is fundamentally an individual's right to decide on their own personal participation in microbial challenge studies based on the risks and rewards (whatever the size of that potential reward, as is the case for anyone working in high risk occupations).

1.3 Periscope consortium

This study is part of work package 2 of the Periscope consortium. The Periscope consortium brings together internationally renowned scientists with many years of experience in *B. pertussis* research, clinical trials, bioinformatics, immunology and public health to promote scientific and technological innovation in pertussis vaccine development and to foster the creation of a laboratory and scientific network that facilitates the testing and helps expedite the development of novel pertussis vaccines in Europe. This study, the development of a human challenge model for *B. pertussis*, is one of the models that will accelerate the development and registration of novel pertussis vaccines and will provide samples for studies performed within the network (see Figure 4).

Figure 4: Overview of studies conducted by Periscope analysing pathogenesis and host response to *B. pertussis*



2. Controlled infection model

2.1 Microbial challenge studies of human volunteers

The deliberate infection of human volunteers with micro-organisms has contributed uniquely to our understanding of the pathogenesis, immune responses and the treatment and prevention of numerous microbial diseases including influenza, cholera, typhoid and hepatitis (43). A review by the UK Academy of Medical Sciences on microbial challenge studies recognised that such studies are desirable for providing proof of concept for prophylactic and therapeutic interventions and can significantly accelerate progress to Phase III studies (43). A human controlled infection model has been used studying various organisms and access to protocols and SOPs has enhanced safety and standardisation as discussed at the Controlled Human Infection Studies in the Development of Vaccines & Therapeutics Wellcome Trust Scientific Conference, January 9-11, 2013, University of Cambridge, UK.

2.2 Ethical considerations of challenge studies

Participants in challenge trials are healthy volunteers who do not obtain direct health benefit from participation. Potential participants will be informed of all conceivable risks and have adequate time to decide on their individual participation in relation to the risks involved and financial compensation for the time and inconvenience of taking part. Medical ethicists have argued that it is a healthy adult's right to self-determine their participation in such trials in relation to the risk (Controlled Human Infection Studies in the Development of Vaccines & Therapeutics Wellcome Trust Scientific Conference, January 9-11, 2013, University of Cambridge, UK).

Challenge trial investigators will exercise all possible safeguards for volunteer and staff safety to ensure that trial participation carries minimal risk. Investigators will also ensure that

1
2
3 maximal scientific benefit accrues from each challenge trial. Key ethical considerations
4 agreed by consensus of the field include: (44)

5
6
7 1. Safety is the paramount consideration in conduct of challenge trials. When challenge trials
8 are conducted, the practical considerations should always be focused on volunteer and staff
9 safety.

10
11 2. Investigators are required to follow both international and local guidelines with respect to
12 ethical considerations and in accordance with the Declaration of Helsinki and should fulfil all
13 local regulatory and research ethics committee requirements.

14
15 3. Challenge trials should be conducted according to WHO Good Clinical Practice
16 Guidelines. The scientific benefit should be maximised whilst minimising risk and
17 discomfort/distress to individuals. From this perspective it is important that the results of
18 challenge trials of *B. pertussis* collect as much information as is reasonably possible about
19 the process of *B. pertussis* colonisation of the human pharynx. Data should be made
20 available to the scientific community to inform the design of future challenge trials and
21 design of *B. pertussis* vaccines.

22
23
24 4. After publication the original data from challenge trial datasets should be made publicly
25 available to facilitate scientific benefit to the community.

26 27 28 **2.3 International guidelines for conducting challenge studies**

29
30 In this study international guidelines will be followed to make sure that the risks to the safety
31 of participants (both of the enrolled subjects and the broader public) is not greater than is
32 acceptable in other forms of research, and that the standards applied to microbial challenge
33 studies of humans is of equivalent stringency to those that pertain, for example, to research
34 on investigational medicinal products.

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36 We have paid particular attention to the following:

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- Transparency and accountability in protocol design, training, trial conduct, safety monitoring procedures and the reporting of adverse events
 - Procedures for the recruitment of volunteers, defining eligibility, compliance, assessing competence, provision of information, and the avoidance of coercion and conflicts of interest
 - Ethical and safety considerations, evaluating the concepts of minimal risk of harm and risk assessment
 - Risk mitigation strategy for secondary transmission and environmental contamination
 - Preparation of microbial challenge materials, including the identification of key elements in quality control and compliance with acceptable production standards (Good Manufacturing Practice (GMP))
 - Good characterisation of the challenge organism

- Well defined rescue therapy initiation criteria, taking into account the sensitivity of challenge strain
- Procedures for consent with demonstration of understanding of risks and confidentiality, including methods for ensuring respect for the autonomy of potential research participants and the use of tissue samples
- Insurance is in place to cover any negligent harm caused within the activities stated in the protocol.

2.4 Southampton NIHR WTCRF governance arrangements for early phase experimental medicine.

As an experimental medicine microbial challenge study, this study does not require MHRA approval. However, the Southampton NIHR-WTCRF has established guidance on governance conduct and standards of experimental medicine work that is in line with that of risk phase I trials of investigational medicinal products.

The Southampton NIHR WTCRF has been granted MHRA accreditation as a phase I Clinical Trials Unit (<https://www.gov.uk/guidance/mhra-phase-i-accreditation-scheme>) and is in the process of reapplying for ongoing accreditation. The NIHR funded the Southampton NIHR-WTCRF to apply for and meet the scheme standards from 2014 as part of the core funding of the unit. It has put several extra safety measures in place to comply with the Phase I Accreditation Scheme.

In line with this similar measures are applied to all high risk experimental medicine research studies, even where no formal Clinical Trial Authorisation is required.

Some of the key safety measures include:

- The Southampton NIHR Wellcome Trust Clinical Research Facility is located within University Hospital NHS Foundation Trust, adjacent to the acute medical and intensive care wards.
- Measures are in place to confirm past medical history of volunteers – either from their GP or hospital consultant.
- The national TOPS scheme is used to prevent duplication or over-recruitment of healthy volunteers to research studies.
- All clinical staff are trained in specific infection prevention measures and PPE use used in this study.
- All clinical nursing staff in the NIHR-WTCRF are Immediate Life Support (ILS) trained (with annual updates) as a minimum.

- All clinical staff attend an emergency scenario training session at least annually.
- Standard Operating Procedures (SOPs) are in place for managing common medical emergencies such as anaphylaxis, syncope etc.
- Participants are provided with 24 hour emergency contact numbers, and these numbers are formally tested out of hours.
- Emergency resuscitation trolleys are in place throughout the facility and the contents are checked on a weekly basis.
- There is a mechanism to notify Intensive Care Unit (ICU) and the emergency response team of all high risk studies, providing study information / dosing schedules, dates of challenge etc.
- Emergency call bells are tested every three months to ensure that they are in working order, and a bi weekly check of pull cords is conducted to make sure they are accessible and have not been tied up or moved out of reach.
- As a hospital ward, we have full use of the 24 hour emergency response teams in case of a medical emergency.

2.5 Public Health England and Infection Prevention Unit at University Hospital Southampton NHS Foundation Trust

This protocol and the safety measures including participant isolation and management of possible *B. pertussis* disease have been developed together with the director of the Infection Prevention Unit at University Hospital Southampton NHS Foundation Trust, following the PHE guidelines for public health management of Pertussis (19) and the Health Protection Agency (HPA) guidelines for the public health management of pertussis incidents in the healthcare setting (45). Measures to prevent *B. pertussis* infection of contacts of the volunteers have been discussed with Dr. Gayatri Amirthalingam, national lead for pertussis, Public Health England.

2.6 Patient and Public Involvement

In the NIHR Wellcome Trust Clinical Research Facility Southampton we have been participating as investigators in several human challenge trials, working together with the Jenner Institute at the University of Oxford.

Feedback from volunteers has been used to refine the design of the study. During the development of the protocol, volunteers participating in other challenge studies have been interviewed about the design of this Pertussis challenge study, with a special focus on the admission and the safety measures that are in the protocol.

A lay update of the study will be posted on the website of Periscope to inform volunteers about the progress of the study. A lay summary will be posted after the trial has been published.

3. Objectives

3.1 Primary objective

To develop a safe controlled human challenge colonisation model with *B. pertussis*

3.2 Secondary objectives

- To establish 70% asymptomatic colonisation of *B. pertussis* in healthy volunteers after nasal inoculation with *B. pertussis*.
- To assess *B. pertussis*-specific immunity in healthy volunteers before and after nasal inoculation with *B. pertussis*
- To assess environmental shedding of *B. pertussis* following nasal inoculation of healthy volunteers with *B. pertussis*.

4. Description and justification of the study design

4.1 Overview

This is a prospective controlled human challenge study consisting of two phases;

Phase A: Development of a *B. pertussis* human challenge model; pilot to establish the standard inoculum

The first aim of phase A is to determine a 'standard inoculum' (SI), which results in safe colonisation of 70% of volunteers. This level of colonisation of 70% (ID70) has been selected so that baseline immune profiles of individuals who are, or are not colonised following challenge can be assessed and biomarkers of protection from colonisation identified. It is acknowledged that for the future use of the human challenge model for efficacy evaluation of experimental vaccine candidates, it would be optimal if the percentage of subjects successfully colonised were at least 70%. The SI will be identified in a dose escalating or de-escalating experiment commencing at 10^3 colony forming units *B. pertussis* administered intranasally (see paragraph 5.9). Each group of 5 volunteers will be sequentially inoculated at half log-fold increasing/decreasing doses until the endpoint is reached. The experiment will be continued until the SI yields 10 subjects who are colonised at day 14. Volunteers will be screened to exclude those with evidence of recent *B. pertussis* infection using anti-PT IgG ELISA as evidence to allow evaluation of seroconversion. Following the challenge chemical, haematological and clinical parameters will be monitored and nasal swab samples will be cultured at regular intervals to assure safety of the volunteers and to identify the presence of *B. pertussis* (see paragraph 8.3). At day 14 after the challenge, or at the onset of symptoms, whichever occurs soonest, eradication therapy in the form of azithromycin 500 mg once a day for 3 days (19) will be given. Further mucosal and blood samples will be taken over the follow up period of one year.

The second aim of phase A is to identify the 'colonisation period'; the earliest day after inoculation at which colonisation of the nasopharynx (as detected by culture) is observed in 100% of those volunteers who show seroconversion at day 28. This time period will be used

1
2
3 to establish the length of participation required from volunteers in future studies. The
4 colonisation period will be deemed biologically relevant if *B. pertussis* specific mucosal and
5 systemic antibodies are elicited in people who are colonised for the colonisation period. A
6 quantitative PCR assay to detect *B. pertussis* in nasopharyngeal samples will be evaluated
7 to determine if this can provide more rapid information in addition to culture.
8
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10 The third aim of phase A is to assess environmental shedding of *B. pertussis* following nasal
11 inoculation of healthy volunteers with *B. pertussis*. These shedding results will be used to
12 determine the length of admission and isolation in phase B. The shedding of *B. pertussis* by
13 challenged volunteers will be assessed using personal aerosol samplers and environmental
14 sampling. Efficacy of eradication therapy will be assessed (see paragraph 8.4).
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17 **Phase B: Development of a modified *B. pertussis* human challenge model**

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19 In phase B the pilot study data from phase A will be used to design a more practical model, if
20 possible conducted partially in an outpatient setting, which will be conditional on safety and
21 transmission evidence. **The final protocol for phase B will be presented as a protocol**
22 **amendment, because it will be based on the standard inoculum and colonisation**
23 **period identified in Phase A.**
24
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27 Volunteers in phase B will not be preselected to exclude those with evidence of recent *B.*
28 *pertussis* infection. The standard inoculum determined in phase A will be used for all
29 volunteers and eradication therapy will be given after the colonisation period based on the
30 data of phase A. Approximately 30 individuals will receive the intranasal SI and as control
31 group approximately 15 individuals will receive intranasal sham. Both groups will be treated
32 with azithromycin for three days at the end of the colonisation period.
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35 Aims:

- 36
37 - To confirm that the following parameters of the model in phase B are similar to that
38 seen in phase A:
39 o Volunteer safety
40 o Colonisation rate
41 o Colonisation period
42 o Genetic/expression changes in *B. pertussis* during challenge
43 o Environmental shedding
44 o Efficacy of eradication therapy
45
46 - To compare the pattern of detection of *B. pertussis* in nasopharyngeal samples by
47 qPCR to that seen in phase A.
48
49 - To assess *B. pertussis*-specific immunity before and after inoculating healthy
50 volunteers with *B. pertussis*, comparing the data from successfully colonised
51 participants with the data from those not colonised and the control group.
52
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54 **4.2 Study volunteers**

55 Healthy volunteers aged 18-45 years will be recruited for both phases. Specific inclusion and
56 exclusion criteria can be found in paragraph 6.4.
57
58

59 Phase A: Volunteers will be excluded from this phase of the study if they have evidence of
60 recent exposure to *B. pertussis*, as determined by anti-PT IgG ELISA (>20 IU/mL). The
experiment will be continued until the SI yields 10 subjects who are colonised by 14 days.

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3 We estimate that we need to screen about 50 volunteers to challenge about 35 eligible
4 volunteers of whom about 18 will be colonised.
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7 Phase B: Volunteers will not be preselected to exclude those with evidence of recent
8 exposure to *B. pertussis*. Approximately 30 individuals will receive the intranasal SI and as
9 control group approximately 15 individuals will receive intranasal sham. The volunteers will
10 be enrolled in the SI group until at least 10 colonised and 10 are not colonised individuals
11 after inoculation. We estimate that we need to screen about 50 volunteers to challenge
12 about 45 eligible volunteers.
13
14

15 **4.3 Randomisation and blinding**

16 There will be no randomisation or blinding in this study. In phase B volunteers are allocated
17 to the SI or sham group according to their preference. This will be asked before screening.
18 Once one of the two groups is full, only volunteers who are interested to participate in the
19 other group will be screened.
20
21

22 **4.4 First volunteers - safety**

23 Phase A: The first volunteer receiving *B. pertussis* will be challenged individually, followed by
24 a safety review including safety blood results and clinical data at day 7 (see safety report
25 form *B. pertussis* challenge study). Providing there are no safety concerns and the safety
26 report form is signed by the PI or CI, a second and third volunteer will be challenged. At day
27 7 of the second and third volunteer a safety review of the first three volunteers will be
28 conducted and providing there are no safety concerns, a fourth and a fifth volunteer will be
29 challenged. After day 17 post challenge of the fifth volunteer a safety report will be written,
30 which will be reviewed within one week by the external safety committee before any further
31 volunteers are challenged. The remaining volunteers will be challenged in groups of a
32 maximum of five. The decision to continue challenging the next volunteer of the following
33 group of five is taken by the CI taking into account the advice of the safety committee and
34 will be put writing before the next volunteer is challenged.
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40 If eradication therapy is given to one volunteer before day 14 due to safety concerns, the
41 next volunteer will be challenged individually. If two or more volunteers out of a group of five
42 receive early eradication therapy due to safety concerns, no new volunteers will be
43 challenged until the data have been reviewed by the external safety committee. The study
44 steering committee will review the available data and propose to the external safety
45 committee what inoculum dose and eradication day will be used for the next volunteers. The
46 study will only be continued after approval by the external safety committee. After each dose
47 escalation of the inoculum dose the same sequence of challenging volunteers individually
48 and in pairs will be followed (see Figure 5).
49
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51
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53 Phase B: Volunteers will be challenged in groups of a maximum of five. A safety report will
54 be written (see safety report form *B. pertussis* challenge study) two weeks after inoculation
55 of the fifth volunteer which will be reviewed within one week by the external safety committee
56 before the remaining volunteers are challenged. If early eradication therapy is given to two
57 volunteers due to safety concerns, no further volunteers will be challenged and the study will
58 be on hold. The study steering committee will then review the available data and propose to
59 the external safety committee what inoculum dose and eradication day will be used for the
60

next volunteers. The study can only be continued after approval by the external safety committee.

Figure 5: Flow schedule showing the number of volunteers and safety reviews



4.5 Duration of volunteer participation

The start of volunteer participation is defined as the screening visit. The end of volunteer participation is defined as the last visit. The duration of involvement in this study from screening will be approximately 56 weeks.

4.6 Definition of the start and the end of the trial

The start of the trial is defined as the date of the first screening of the first volunteer. The end of the trial is defined as 12 months after the date of the last visit of the last volunteer, to allow testing of the samples.

4.7 Potential benefits for the volunteers.

It is possible that taking part in this study will result in the participant having a degree of immunity to whooping cough, but we cannot be certain that the volunteer will benefit directly from this study. Volunteers will receive information about their general health status.

4.8 Involvement of the stakeholders in the making of the protocol

Safety monitoring and treatment regimens in the protocol have been reviewed by the external safety committee, the study advisory committee, the scientific advisory board of Periscope, the ethical advisory board of Periscope, and the IMI. After reaching consensus the definitive protocol will be approved by the HRA, REC and local R&D prior to study commencement.

5. Inoculum

5.1 Selection of the *B. pertussis* strain

The *B. pertussis* isolate to be used in this human colonisation model is strain B1917, which is representative of current isolates in Europe (Bart et al., 2015). The strain, isolated in 2000 from a Dutch patient with *B. pertussis* disease, is characterised by *ptxP3-ptxA1-prn2-fim3-2, fim2-1* MLVA27, PFGE BpSR11 and expresses Prn, Ptx and FHA. This strain has been extensively characterised in the mouse model as well as by proteomics and transcriptomics and has a closed genome available. It is fully sensitive to azithromycin in vitro.

5.2 Manufacturing of the *B. pertussis* inoculum

The inoculum will be prepared by Q Biologicals (Ghent, Belgium) to GMP standard in licensed cGMP facilities and using a process free of animal-derived products. The identity and purity of the cell bank will be confirmed, in addition to any other quality specifications agreed with the consortium and needed for compliance with regulatory requirements. There will be no culture of the challenge inoculum at the clinical site, other than to assess the dose and purity of the inoculum after inoculation and quality assessment. The inoculum will be supplied in vials containing 1.1 ml of 10^6 cfu/mL.

5.3 Quality assessment of the inoculum

Of each 10 vials one will be thawed and tested for culture on *Bordetella* selective medium (charcoal blood agar with cephalexin) for determination and viable counts of *B. pertussis* and on a Plate Count Agar to assess purity of the inoculum. Culture identity will be confirmed by visual appearance of colonies and Gram stain. Full molecular identification using for example, PCR and Matrix Assisted Laser Desorption Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS) will be used to identify isolates to species level. UK standards for identification of *Bordetella* species (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/372648/B_6i8.pdf) will be followed.

5.4 Transport of the inoculum

The inoculum will be transferred from Q Biologicals to the NIHR-WTCRF under temperature-monitored conditions. For transport within the NIHR-WTCRF containers of inoculum will be placed into secondary containers, which will be transported in leak and shock resistant transport boxes with secured lids.

5.5 Storage of the *B. pertussis* inoculum

The cell banks will be stored at -80°C ($\pm 20^{\circ}\text{C}$) in a locked, dedicated, temperature monitored freezer in the NIHR-WTCRF.

5.6 Dilution of the inoculum

One vial of inoculum will be removed from the freezer, thawed and diluted to the required inoculum dose in two aliquots of 500 μL following the study specific SOP: Dilution and monitoring of *B. pertussis* inoculum dose. Dilution will be carried out by one staff member and checked by another member. The inoculum will be administered to the volunteer within an hour of removal from the freezer following the specific SOP: Administering the *B. pertussis* inoculum to healthy volunteers.

5.7 Monitoring of the *B. pertussis* dose given to the volunteer

After the inoculum is administered the tube residuum will be diluted and cultured for 5 days on *Bordetella* selective medium (charcoal blood agar with cephalixin) for determination and viable counts of *B. pertussis* and on a Plate Count Agar to assess purity of the inoculum. If the inoculum measured at day 5 is more than 5 times the inoculum intended for the volunteer they will be given oral Azithromycin 500 mg once a day for 3 days, observed for 48 hours in the NIHR-WTCRF and excluded from the study. Safety follow up will take place at day 14, 28 and week 26.

5.8 Disposal of the inoculum

All materials used during the challenge, including any remaining inoculum will be disposed following NHS trust policy.

5.9 Finding the optimal and safe dose of the standard inoculum: Phase A

Providing there are no safety concerns the standard inoculum (SI) will be identified in a dose escalating or de-escalating experiment commencing at 10^3 colony forming units administered intranasally, following the safety schedule described in chapter 4.4 aiming for 70% colonisation of challenged volunteers. The initial low dose is selected based on the previous human challenge studies with *B. pertussis* (17, 39), taking into account we will inoculate healthy previously exposed volunteers. The colonisation rate will be reviewed after five volunteers to adjust the inoculum dose for the next five volunteers. **Colonisation is defined as detection of *B. pertussis* by classical microbiology from nasal wash samples at any time-point.**

Increasing the dose

If no volunteers are colonised at day 14 the inoculum dose will be increased by 1 log. If one or two of five volunteers are colonised then the inoculum dose will be increased by $\frac{1}{2}$ log. If three or four volunteers are colonised, another five volunteers will be challenged with the same dose. If less than a total of 7 volunteers of 10 are colonised, the inoculum dose will be increased by $\frac{1}{2}$ log (see Figure 6 and Figure 7).

Figure 6: Summary of challenge sequence and increasing inoculation dose.

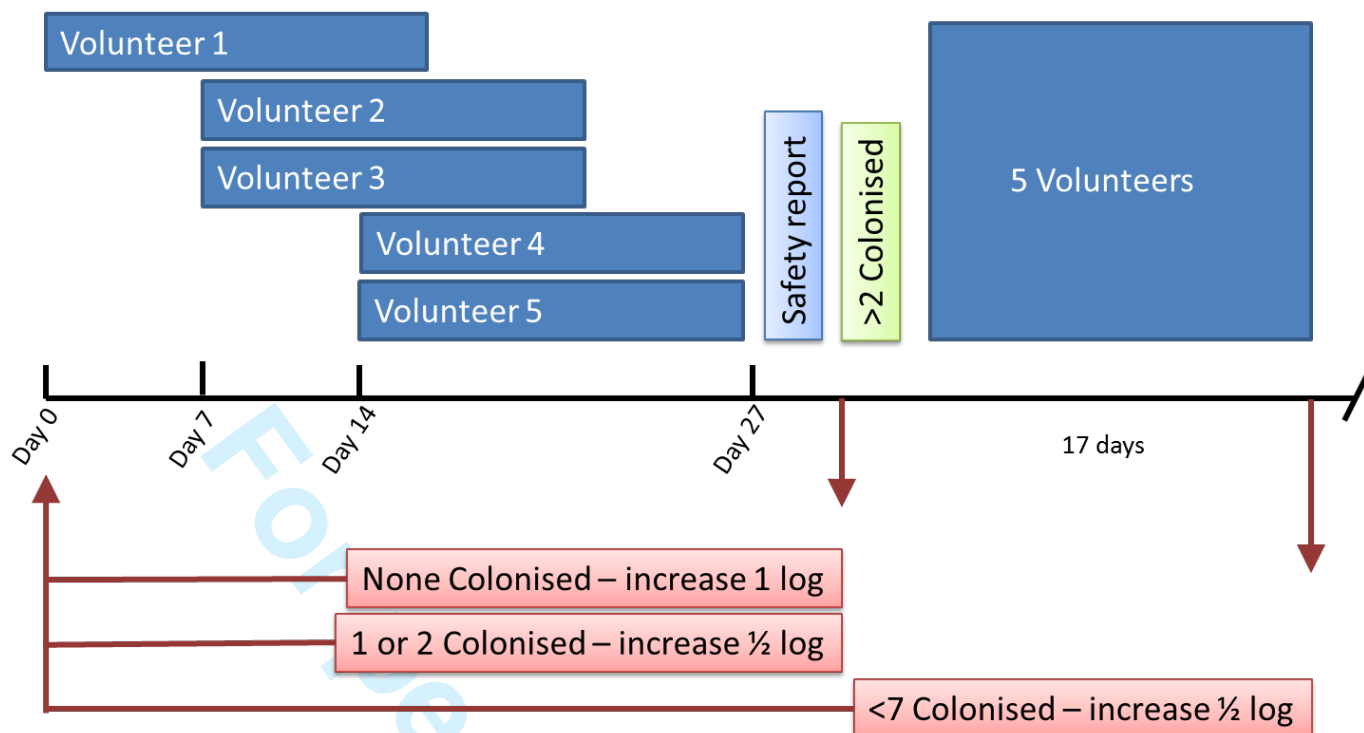
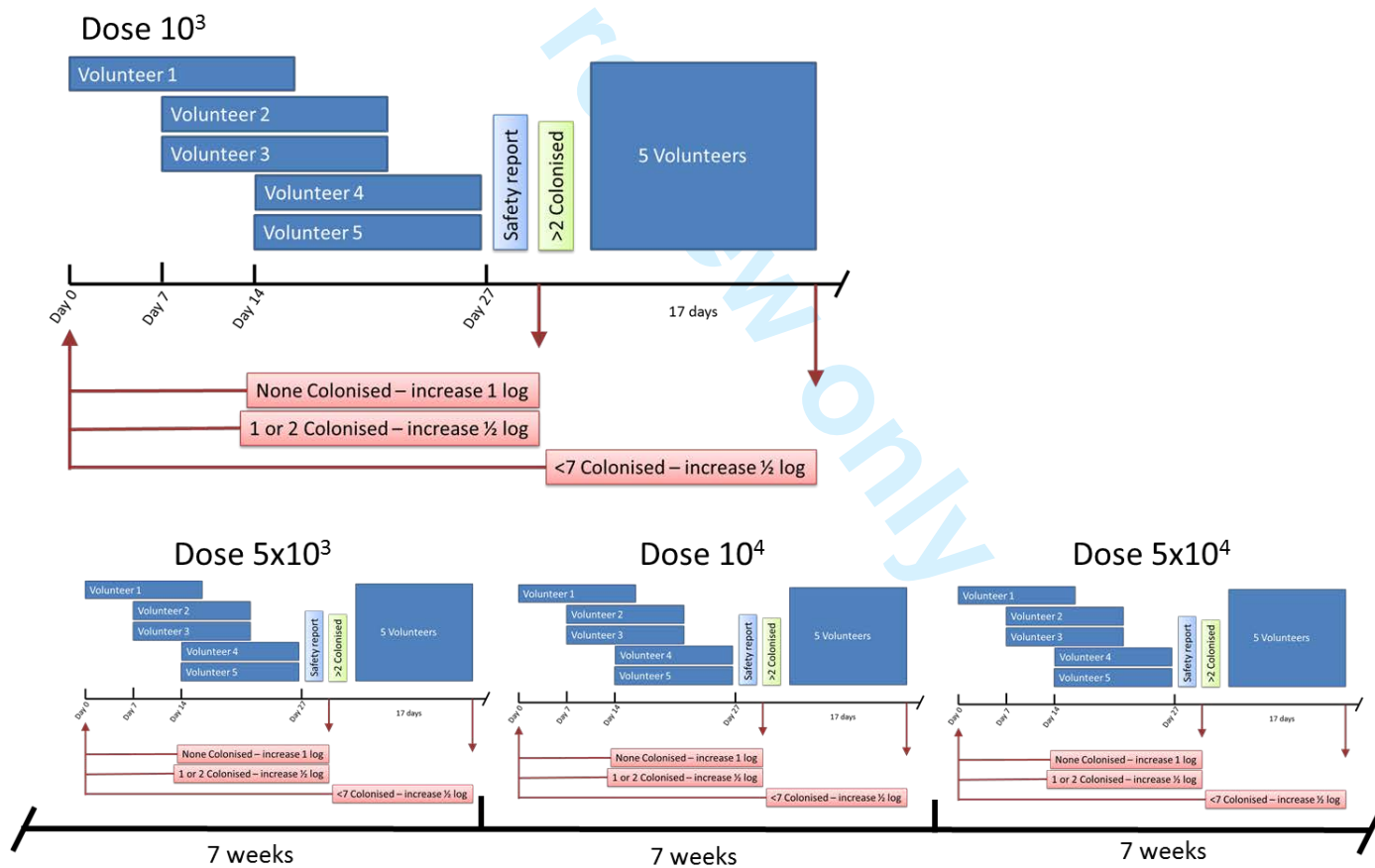


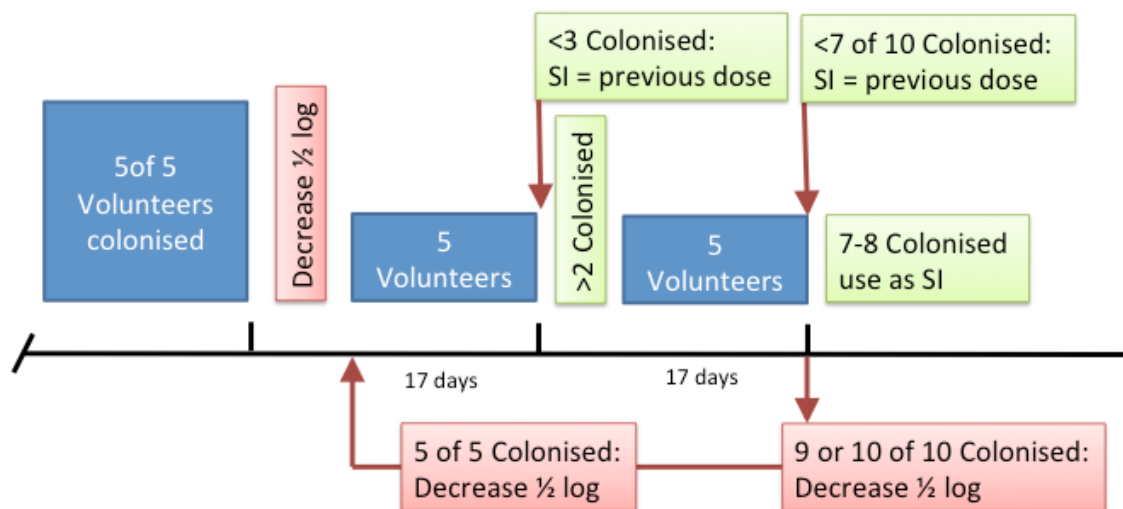
Figure 7: Challenge sequence and increasing inoculation dose.



Decreasing the inoculum dose

If five volunteers are colonised after challenging five volunteers, or nine or ten after challenging 10 volunteers, the inoculation dose will be decreased by $\frac{1}{2}$ log and the schedule shown in Figure 8 will be followed. If the decreased inoculum dose causes less than 60% colonisation, the previous dose will be used as standard inoculum.

Figure 8: Summary of challenge sequence and decreasing inoculation dose.



The experiment will be continued until one dose of inoculum yields 10 subjects who are colonised within the 14 days. This dose will be considered as the standard inoculum and will be used as the standard inoculum in phase B.

6. Recruitment of trial volunteers

6.1 Recruitment

Recruitment and screening will commence approximately one month prior to the first challenge. Healthy volunteers aged 18-45 will be recruited through various media. Care will be taken not to recruit from vulnerable groups (mental health or other capacity issues or those under 18 years old). This will be checked during screening. The recruitment strategy will be developed in conjunction with the NIHR-WTCRF Project Management team, which has vast experience in recruiting healthy volunteers for complex/high risk studies and will be approved by the ethical committee and the HRA. Prior to the commencement of the study detailed information will be provided to the IMI and the Periscope consortium on the procedures that will be used for the recruitment of participants (e.g. number of participants, inclusion/exclusion criteria, direct/indirect incentives for participation, the risks and benefits for the participants etc.).

Volunteers may be recruited by use of an advertisement +/- registration form formally approved by the ethics committee and distributed or posted in the following places:

- On the website of Periscope where information will be given and the volunteer information sheet will be downloadable

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

6.2 Volunteer information sheet

A volunteer information sheet will be available on the study website and will be given to potential volunteers at least 24 hours before the screening visit. The volunteer information sheet will include all risks and safety measures that are involved in the study. It will be reviewed by the external safety committee and send to the IMI, scientific advisory board of Periscope, ethical advisory board of Periscope and formally approved as part of the HRA application and R&D.

6.3 Screening visit (Day-30)

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

6.3.1 Pre consent questionnaire

After their understanding about the study aims and level of involvement required has been established and if they wish to participate, volunteers will be asked to complete a questionnaire testing their understanding of the trial. Volunteers who fail to answer all questions correctly on their first attempt will be allowed to re-take the questionnaire following further discussion with the investigator. If the volunteer is not able to answer all questions correctly within three attempts, he/she will be asked to read the patient information sheet again and come back at least 24 hours later for a screening visit and repeat the pre-consent questionnaire.

6.3.2 Informed consent

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

- Participation in the study is entirely voluntary
- Refusal to participate involves no penalty or loss of medical benefits
- The volunteer may withdraw from the study at any time. However, if the volunteer has received the *B. pertussis* inoculation and not completed a course of appropriate antimicrobial therapy then the volunteer is strongly advised to maintain contact with the investigators for monitoring and treatment
- The volunteer is free to ask questions at any time to allow him or her to understand the purpose of the study and the procedures involved
- Other than potential immunity against future pertussis, there is no direct benefit from participation
- The volunteer's GP will be contacted to corroborate their medical history and confirm that the volunteer is eligible to take part in the study. Volunteers will only be enrolled in the study if written or verbal information regarding the volunteer's medical history is obtained from the GP
- The volunteer will be registered on the TOPS database (The Over-volunteering Prevention System; www.tops.org.uk)
- The aims of the study and all tests to be carried out will be explained. The volunteer will be given the opportunity to ask about details of the trial, and will then have time to consider whether or not to participate
- Blood tests can include some genetic tests (participation in this study will not be affected by the decision to allow or not allow genetic tests to be carried out)
- The isolation procedures will be explained (see paragraph 8.2.3) and its importance will be emphasised.
- The risks of participating in the study will be fully explained
- Study samples and data will be shared with other institutions within the EU, investigating the immune response to *B. pertussis*
- Study samples and data will be stored for possible future studies investigating the immune response to *B. pertussis*
- Volunteers will be given the option to be contacted in the future for possible follow up trials looking at pertussis

Provided the volunteer answers all questions in the questionnaire correctly, they will be asked to sign and date two original copies of the consent form, which will be signed and dated by the investigator. One original copy is for them to keep, one will be stored in the CRF in the NIHR-WTCRF. A copy of the original will be kept in the patient's medical notes. They may then be screened for the trial.

6.3.3 Medical history and physical examination

A detailed medical history and screening physical examination will be conducted by the investigator to ensure the volunteer meets all inclusion and no exclusion criteria. The General Health Questionnaire (GHQ) (46) will be used as a screening device for identifying personality or psychiatric disorders that might make it harmful to the individual to take part in

1
2
3 the study. If abnormal results or undiagnosed conditions, e.g. anaemia or a psychological
4 disorder, are found in the course of the study these will be discussed with the volunteer and
5 their GP will be informed.
6
7
8
9

10 **6.3.4 Screening bloods, urine, ECG and nasal swabs**

11 Screening bloods, urine, nasal swabs and electrocardiogram (ECG) will be taken according
12 to the protocol (see Table 2). Females will have a pregnancy test.
13

14 Taking blood samples, urine samples, nasal samples and making an ECG will be done
15 following local SOPs.
16

17 **6.4 Inclusion and exclusion criteria**

18 **6.4.1 Inclusion criteria**

19 The volunteer must satisfy all the following inclusion criteria to be eligible for the study:
20

- 21 • Healthy adults aged 18 to 45 years inclusive on the day of screening
- 22 • Fully conversant in the English language
- 23 • Able to communicate easily by both mobile telephone and text messaging
- 24 • Able and willing (in the investigator's opinion) to comply with all study requirements
- 25 • Written informed consent to participate in the trial
- 26 • Willingness to take a curative antibiotic regimen after inoculation with *B. pertussis*
27 according to the study protocol
- 28 • Agreement to be admitted to the NIHR-WTCRF Southampton for 17 days for phase
29 A (from inoculation until two days after the eradication therapy is given) and for the
30 duration necessary for phase B, depending on phase A results
- 31 • Able to answer all questions on the informed consent quiz correctly
32

33 **6.4.1 Exclusion criteria**

34 The volunteer may not enter the study if any of the following criteria apply:
35

- 36 • Individuals who have inviolable commitments within 3 months of discharge from the
37 inpatient phase of the study to make contact with:
 - 38 a. unimmunised or partially immunised children and infants aged < 1 year
 - 39 b. pregnant women >32 weeks who have not received pertussis vaccination at
40 least a week prior to contact
- 41 • Individuals who have household contacts working with
 - 42 a. unimmunised or partially immunised children and infants aged < 1 year
 - 43 b. pregnant women >32 weeks who have not received pertussis vaccination at
44 least a week prior to contact
- 45 • Phase A only: Volunteers will be excluded from this study if they have evidence of
46 recent exposure to *B. pertussis*, as determined by anti-PT IgG ELISA (>20 IU/mL)
- 47 • *B. pertussis* detected on nasopharyngeal swab taken before the challenge
- 48 • Individuals who have a signs of a current infection at the time of inoculation with *B.*
49 *pertussis*
- 50 • Individuals who have participated in other interventional clinical trials in the last 12
51 weeks
52

- Individuals who have a history of receiving *B. pertussis* vaccination in the last 5 years
- Individuals who have a history of never being vaccinated against *B. pertussis*
- Current smokers defined as having had a cigarette/cigar in the last week.
- Use of systemic antibiotics within 30 days of or during the challenge
- Any confirmed or suspected immunosuppressive or immune-deficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) immunosuppressant medication within the past 6 months (topical steroids are allowed)
- Use of immunoglobulins or blood products within 3 months prior to enrolment
- History of allergic disease or reactions likely to be exacerbated by any component of the inoculum
- Contraindications to the use of azithromycin or macrolides
- Pregnancy, lactation or intention to become pregnant during the study (see paragraph 6.4.2)
- Any clinically significant abnormal finding on biochemistry, haematology, toxicology or serological blood tests, urinalysis (see Table 2) or clinical examination - in the event of abnormal test results, confirmatory repeat tests will be requested
- Any other significant disease, disorder, or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data, for example recent surgery to the nasopharynx

6.4.2 Effective contraception for female volunteers

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

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

6.5 Screening tests

Table 2: Screening bloods and exclusion criteria

Biochemistry	Lower limit	Upper limit
C reactive protein [mg/l]	N/A	7.5
Potassium [mmol/L]	3.5	5.3
Sodium [mmol/L]	133	146
Urea [mmol/L]	N/A	7.8
Creatinine [μ mol/L]	N/A	97
Albumin [g/L]	35	N/A
Total bilirubin [μ mol/L]	N/A	20

ALT [IU/L]	N/A	40
ALP [IU/L]	N/A	130
Haematology		
Haemoglobin [g/L]	Male: 130, Female: 120	Male: 170 Female: 150
White Cell Count [x 10 ⁹ /L]	4	11
Neutrophil count [x 10 ⁹ /L]	2	7.5
Lymphocyte count [x 10 ⁹ /L]	1.5	4.0
Platelet Count [x 10 ⁹ /L]	150	400
Serum		
Phase A: anti-PT IgG ELISA IU/mL		>20
Toxicology screening		Positive
Urinanalyses		
Protein		2+ or 0.5-1gm loss/day
Blood		2+ confirmed by 5-10 rbc/hpf
Glucose		1+
Pregnancy test		Positive
Nasopharyngeal swab		
Culture		<i>B. pertussis</i>
ECG		
QTc		≥440 ms.

Lower and upper values of normal in blood tests, urine test and nasopharyngeal swab are based on normal values Southampton University Hospital NHS Foundation Trust Clinical Laboratory

6.6 Pre-challenge visit (Day -7)

Once the GP letter confirming eligibility of the volunteer is received, the pre-challenge visit is booked. A nasopharyngeal swab will be performed to ensure the volunteer is still not colonised and nasal samples will be taken for baseline measurements. If the nasopharyngeal swab is still negative it will be assumed that the volunteer is not colonised on the challenge day.

Throat swab and saliva sample

A throat swab will be collected following the local SOP

Nasal wash

The nasal wash will be done using the method described by Naclerio et al (46) and specified in a SOP: Collecting a nasal wash sample. Nasal washing and PCR provide effective alternatives to nasopharyngeal swabbing and classical microbiology, respectively (47). The same study showed that 91% of volunteers found nasal wash to be more comfortable than nasopharyngeal swab. Nasal washes will be analysed to allow a quantitative measurement of the colonisation density.

7. *B. pertussis* challenge (Day 0)

7.1 Trial site

The challenge will take place in the NIHR Wellcome Trust Clinical Research Facility at UHS NHS FT. Relevant facilities include patient/volunteer consultation, waiting and recreation areas; 13 consulting rooms (2 configured for infectious participants); a containment level 3 laboratory for microbiological work, 8 in-patient beds; an environmental laboratory with three “containment level 2” environmental chambers; state-of-the-art physiological monitoring and physical and management systems to ensure Regulatory Compliance such as computerised sample inventory, and tracking system

(<http://www.uhs.nhs.uk/ClinicalResearchinSouthampton/Trials-and-facilities/NIHRWellcome-Trust-Clinical-Research-Facility/Our-facility.aspx>). The volunteers will have designated areas including rooms, toilets, shower and recreational room during their stay at the facility. Standard infection control precaution policy will be followed as per NHS and PHE policy.

7.2 Clinical team involved in the challenge

The challenge will be conducted by the study doctor, together with a study nurse. The study doctor will be responsible for the administration of the inoculum. Because this is a First in Human (FIH) study, full Advanced Life Support (ALS) trained medical and nursing cover will be present on inoculation, 24 hours a day two staff members will be present in the unit to review the volunteers at any time and the study doctors will be on call 24 hours/day during the study. Presence of staff will be logged. All study staff will have appropriate training in infection prevention and use of PPE. The NIHR-WTCRF is situated in the University Hospital Southampton NHS Foundation Trust and a resuscitation team and intensive care facilities are available. Volunteers would have a 24 hour contact number to contact the PI and research team in case of any adverse reactions during the study.

The dose of the inoculum will be prescribed on a study-specific prescription form and supplied to the laboratory preparing the inoculum.

7.3. Challenge Procedures

7.3.1 Confirmation of identity of the volunteer – Monday 8.00

The identity of the volunteer will be confirmed using photo ID and TOPS. The study ID from the medical file will be checked to the case report file, the label on the inoculum request form and the blood request forms. The volunteer will get an ID wristband, conform the NHS policy for admitted patients.

7.3.2 Review before the challenge – Monday 9.00

Before any procedure is performed, the study team will check that the safety reviews have been signed and permission to continue challenging the next volunteer has been obtained from the PI/CI. The volunteer will be asked if he/she has any questions about the study and agrees to continue with the study. Eligibility will be confirmed before the challenge is conducted.

Medical history

The investigator will take a medical history asking if there are any new medical issues since the screening visit. Special attention will be given to any possible signs of infection such as a coryzal symptoms or fever. If any abnormalities are found, the challenge will be postponed.

Physical examination

A physical examination will be conducted to check for any abnormalities. Vital signs like heart rate, blood pressure, respiratory rate and temperature will be recorded. If there are any abnormalities found, the challenge will be postponed.

Laboratory investigations

A blood sample will be taken for safety bloods, baseline immunology and HLA and a throat swab for a respiratory virus PCR panel will be done. A pregnancy test (urine test) will be performed in female volunteers. If the pregnancy test is positive the challenge will be cancelled.

7.3.3 Challenge – Monday 11.00

Members of the study team present at the challenge

The challenge will be conducted by the study doctor, together with a study nurse. In the first 3 volunteers the principal investigator will be present at the challenge.

Place of the challenge

The challenge will take place in a designated area inside the NIHR-WTCRF.

Time schedule of the challenge

The inoculation will take approximately 5 minutes, after which the volunteer will remain in the room for 15 minutes of close observation. After this period of observation the volunteer will be brought to his/her room in the NIHR-WTCRF for further observation.

Preparing the inoculum

The inoculum will be prepared in the NIHR-WTCRF laboratory by technical staff using a dedicated containment level 2 safety hood following the study-specific SOP: preparation and dilution of the *B. pertussis* inoculum for human challenge.

Administering the inoculum

The inoculum will be administered in the NIHR-WTCRF by the investigator following the study-specific SOP: Administering the *B. pertussis* inoculum to healthy volunteers.

Preparing and administering the sham inoculum

The sham inoculum will be prepared and administered following the study-specific SOP: preparation and administration of the sham inoculum in human challenge studies, taking care not to allow cross-contamination with the volunteers receiving the *B. pertussis* inoculum.

Containment of *B. pertussis* during the challenge

The challenge procedure will be carried out in one of the containment level 2 environmental chambers within the NIHR-WTCRF to assure the inoculum will be administered to the volunteer only, without posing any risk of infection to other people or the environment. Staff will wear appropriate personal protective equipment during the challenge procedure as shown in Table 3. Before and after the challenge they will wash their hands and use alcohol to clean their hands. After the challenge the room will be cleaned following NHS guidelines.

Table 3: Personal protective equipment (PPE).

	Contact > 2 metres	Contact within two metres	Aerosol-generating procedures
Hand hygiene	✓	✓	✓
Gloves	X	✓	✓
Plastic apron	X	✓	✓
Gown	X	X	✓
Surgical mask	✓	X	X
FFP3 respirator	X	✓	✓
Eye protection	X	✓	✓

Taking nasal fluid, saliva or blood samples is considered contact within 2 metres. The challenge itself and taking nasopharyngeal or throat swabs are considered an aerosol generating procedure.

Disposal of materials

All used material will be disposed of in waste bags and processed following NHS guidelines.

Reporting of procedures

Procedures will be reported in the case report file (CRF).

8 Clinical and laboratory monitoring

After the challenge the volunteers will be admitted to the NIHR-WTCRF to be monitored. Once discharged volunteers will be instructed to call the 24 hour emergency telephone number in case of any concerns or systemic symptoms.

8.1 People involved in monitoring

The clinical team involved in the monitoring of the volunteer will consist of the clinical investigators, NIHR-WTCRF research fellows and research nurses.

The NIHR-WTCRF SOPs prevent staff lone working and require at least two members of staff to be present for overnight studies on the unit. Two nurses will be on duty overnight, a research physician will be on call (according to UHS NHS rules for acute medical consultants on call, able to attend unit within 30 minutes of urgent call). The NIHR-WTCRF is

1
2
3 treated as a hospital ward so is covered by the hospital emergency response teams and
4 medical intensive care support 24 hours per day.
5

6 7 **8.2 Containment of *B. pertussis* during admission**

8 9 **8.2.1 Identifying the 'colonisation period'**

10 **The colonisation period is defined in phase A as the earliest day after inoculation at**
11 **which colonisation of the nasopharynx (detected by culture) is observed in 100% of**
12 **the volunteers who show seroconversion at day 28.** To determine this period regular
13 post nasal washes (culture) will be taken (see Table 4 and Table 5). The colonisation period
14 will be used in phase B to shorten the length of participation and admission required from the
15 volunteers.
16

17
18 The colonisation period will be deemed biologically relevant if this colonisation duration
19 elicits anti-*B. pertussis* mucosal and systemic antibodies in Phase B. A quantitative PCR
20 assay to detect *B. pertussis* in nasopharyngeal samples will be evaluated to determine if this
21 can provide more rapid additional information to culture.
22

23
24 In phase A volunteers will be admitted for 17 days. Depending on results from phase A the
25 study steering committee will propose to the external safety committee reducing the length of
26 admission in phase B (in this protocol estimated 7 days).
27

28 29 **8.2.2 Shedding of *B. pertussis* after challenge and eradication.**

30 Nasal washes and nasopharyngeal swabs will be taken in one of the containment level 2
31 environmental chambers within the NIHR-WTCRF facility. The shedding of *B. pertussis* by
32 challenged volunteers will be assessed daily after challenge following the study-specific
33 SOP: assessment of environmental shedding of *B. pertussis* during the human challenge
34 study. This will include analyses of the face mask, sampling the air in the room, taking
35 samples from surfaces in the room, dipping fingertips in a small dish with water and
36 coughing/talking inside a coughbox. The effect of eradication on shedding will be analysed
37 by continuing the shedding assessment for 3 days following eradication. Depending on the
38 shedding results from phase A the level of containment required for participants in phase B
39 and future studies will be proposed by the study steering committee to the external safety
40 committee and the regional ethics committee.
41
42

43 44 **8.2.3 Isolation of volunteers**

45 The volunteers will be admitted to a designated area in the NIHR-WTCRF facility will have
46 access to dedicated rooms, toilets, shower and recreational area during their stay in at the
47 facility. Volunteers are allowed to leave the designated area during daytime for a maximum
48 of two hours twice a day, and are expected to stay inside from 18.00 till 8.00. When they
49 leave the designated area, they will be escorted by a member of the study team. When they
50 come back to the unit, they will be escorted again by a staff member. Meals, drinks, snacks
51 and entertainment will be provided.
52

53
54 To protect the volunteers from developing illness outside the NIHR-WTCRF, to prevent
55 possible cross infection of other people and to protect the staff working in the NIHR-WTCRF
56 and NHS University Hospital of Southampton the volunteer will have to adhere to the
57 following rules:
58
59
60

- The volunteer is not allowed to leave the NIHR-WTCRF without permission of the clinical team during admission
- Volunteers are allowed to leave the designated area during daytime for a maximum of two hours twice a day
- When the volunteer leaves his personal room in the isolation area at the NIHR-WTCRF unit he/she will have to wear a surgical mask to cover his/her nose and mouth.
- When the volunteer is in the recreational room he/she will have to wear a surgical mask to cover his/her nose and mouth.
- The volunteers are not allowed to enter the rooms of other volunteers.
- When the volunteer leaves or comes back the isolation area at the NIHR-WTCRF unit he/she will need to be escorted by a member of the study team.
- He/she is not allowed have contact with
 - a. unimmunised or partially immunised children and infants aged < 1 year.
 - b. pregnant women >32 weeks who have not received pertussis vaccination at least a week prior to contact
 - c. healthcare workers working with vulnerable children or pregnant women
- The volunteer needs to wash his/her hands before leaving his room and is not allowed to have direct face-to-face contact (< 2 metre distance) for greater than a cumulative period of 1 hour with other people during the admission period.
- The volunteer is not allowed to have any direct contact that could involve transfer of respiratory secretions to anyone during the admission period.
- The volunteer is not allowed to use the main entrance of the hospital and the shops and cafe's in the hospital buildings
- When the volunteer leaves the unit he/she must be contactable by mobile phone, which has the study emergency phone number programmed in, and contact the clinical study team if necessary.
- The volunteer must be able to be return to the NIHR-WTCRF within 30 minutes.
- The volunteer may receive a maximum of two guests at a time between 8.00 and 22.00, who must wear masks covering nose and mouth while in close proximity to the volunteer and must adhere to strict infection control procedures.

These rules will be emphasised during the informed consent process. If the volunteer is not following the instructions of the clinical team and is therefore compromising their own safety and that of the staff or environment, the challenge will be stopped and eradication therapy will be given. If a volunteer is excluded from the study prior to its completion they will be offered financial re-imburement corresponding to the number of visits attended and days admitted.

Within 3 months of discharge from the inpatient phase of the study challenged volunteers will need to avoid contact with:

- a) unimmunised or partially immunised children and infants aged < 1 year
- b) pregnant women >32 weeks who have not received pertussis vaccination at least a week prior to contact
- c) healthcare workers working with vulnerable children or pregnant women

8.2.4 Protection of health workers involved in monitoring

All health care workers involved in this study will be vaccinated against *B. pertussis* at least 2 weeks before working with volunteers that have received the inoculum unless they have been vaccinated against *B. pertussis* in the last 5 years (48). Personal protection equipment will be provided for NIHR-WTCRF staff (see table 9.1). Staff who are >32 weeks pregnant are not allowed to have volunteer contact during admission. Staff in the NIHR-WTCRF who are in contact with participants will be requested to be monitored for carriage of viable *B. pertussis* while volunteers are resident in the NIHR-WTCRF.

8.3 Monitoring safety of volunteers

During admission the volunteers will be reviewed six times per day between at 8.00 AM, 12 AM, 4 PM, 8 PM, 12 PM, and 4.00 AM following a standardised checklist including body temperature, respiratory rate, heart rate and early symptoms of pertussis: rhinorrhoea, nasal congestion, epistaxis, sneezing, ear pain, eye pain, sore throat, cough, dyspnoea, feeling generally unwell, tiredness and headache. Bloods, nasal washes, nasosorption fluid samples and nasopharyngeal swabs will be taken at 9.00 AM as detailed in Table 4 and Table 5. Safety bloods include CRP and FBC.

Table 4: Summary of monitoring procedures during the study phase A.

	D-30 Screening	D-7 Mon	D0 Mon	D1 Tue	D2 Wed	D3 Thu	D4 Fri	D5 Sat	D6 Sun	D7 Mon	D8 Tue	D9 Wed	D10 Thu	D11 Fri	D12 Sat	D13 Sun	D14 Mon	D15 Tue	D16 Wed	W4 FU	W8 FU	W2 6 FU	W5 2 FU
Visit window (days)	- 7	± 1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	± 3	± 7	± 14	± 14
Visit	x	x																		x	x	x	x
Admission			x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x				
Challenge			x																				
Bloods	x		x	x	x	x			x	x		x		x			x			x	x	x	x
Urine	x*		*																				
ECG	x																						
Nasal wash		x					x			x		x		x			x	x	x	x	x	x	x
Nasosorption fluid		x					x			x		x		x			x	x	x	x	x	x	x
Nasopharyngeal swab	x	x				x		x		x		x		x			x	x	x				
Saliva sample		x				x		x		x		x		x			x	x	x				
Throat swab		x				x		x		x		x		x			x	x	x				
Respiratory PCR panel			x	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)	(x)							
Azithromycin																	x	x					
Environmental samples				x	x	x	x	x	x	x	x	x	x	x	x	x	x	x					
Safety bloods (ml)	8		8		8	8	8		8	8		8		8			8		8				
Immunology bloods (ml)	10		60	20		20	20			50		20		20			50			60	60	60	60
Cumulative blood volume (ml)	18		86	106	114	142	170		178	236		264		292			350			418	486	546	606

(x): On indication, * pregnancy test for females

Table 5: Proposed summary of monitoring procedures during the study phase B with eradication day on day 7.

	D-30 Screening	D-7 Mon	D0 Mon	D1 Tue	D2 Wed	D3 Thu	D4 Fri	D5 Sat	D6 Sun	D7* Mon	D8 Tue	D9 Wed	W4 FU	W8 FU	W26 FU	W52 FU
Visit window (days)	- 7	± 1	0	0	0	0	0	0	0	0	0	0	± 3	± 7	± 14	± 14
Visit	x	x	x				x			x*			x	x	x	x
Admission ^(a)			x	x	x	x	x	x	x	x						
Challenge with SI or sham Inoculum			x													
Bloods	x		x				x			x			x	x	x	x
Urine	x*		*													
ECG	x															
Nasal wash		x					x			x			x			
Nasosorption fluid		x					x			x			x			
Nasopharyngeal swab	x	x					x			x			x			
Saliva sample		x					x			x			x			
Throat swab		x					x			x			x			
Respiratory PCR panel			x	(x)	(x)	(x)	(x)	(x)	(x)							
Azithromycin										x	x	x				
Environmental culture				x	x	x	x	x	x	x						
Safety bloods (ml)	5		5				5			5			5	5		
Immunology bloods (ml)			60				30			60			60	60	60	60
Cumulative blood volume (ml)	5		70				105			170			235	300	360	420

(a) SI group only, (x): on indication, * pregnancy test for females

Note: A final schedule will be submitted to the REC as a substantial amendment based on the results of phase A.

8.4 Eradication therapy (phase A: day 17, phase B: tbc)

To ameliorate risk of transmission of *B. pertussis* to the environment and household contacts azithromycin 500 mg once a day for 3 days will be given to eradicate colonisation with *B. pertussis*. The inoculum strain is fully sensitive to this antibiotic. Previous studies show that azithromycin eradicates colonisation in 97% of people in 48 hours. Although no data are available we expect that the colonisation density will be decreased by azithromycin in the remaining 3%. This will be confirmed by data collected in phase A from quantitative measurement of cultures from nasal wash samples on day 15, 16 and day 17. Transmission is expected to be lower in individuals without any symptoms reducing the risk. It will be emphasised that contact rules will need to be adhered to until the nasopharyngeal culture taken 2 days after eradication therapy (day 16) is negative for *B. pertussis*. If the day 16 culture is positive, an extra nasopharyngeal culture will be taken on the day the day 16 culture becomes positive and another course of azithromycin will be given. A nasopharyngeal culture will be repeated on day 3 of the course. This will continue until the nasopharyngeal culture is negative. In case initial eradication therapy is unsuccessful the PHE consultant in communicable disease control covering the area of residence of the volunteer will be informed so that prophylactic treatment can be offered to household contacts of this volunteer if considered necessary.

Eradication therapy will be given at the end of each experiment, or following withdrawal of the volunteer from the study.

The eradication day in phase B will be determined after review of the data of phase A.

8.4.1 Compliance with the eradication therapy

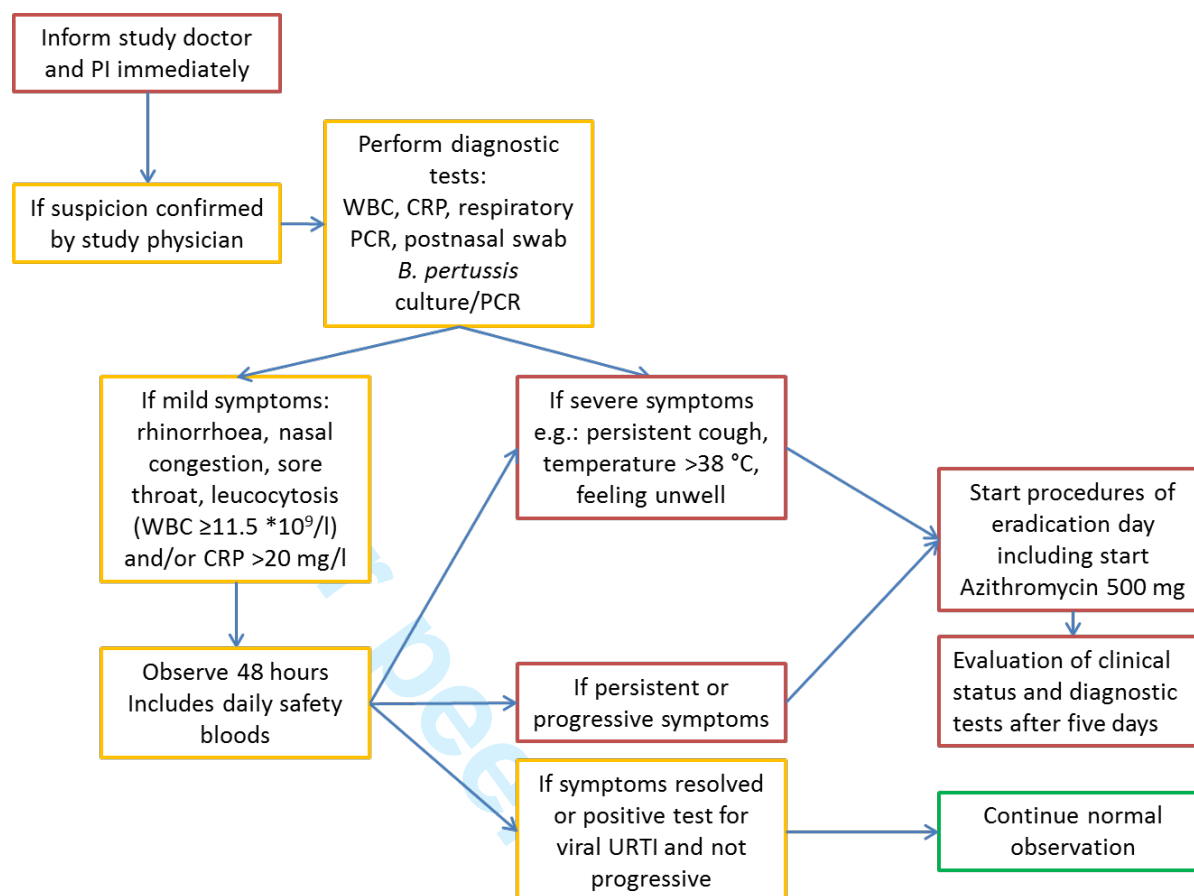
The doses will be taken under supervision of the study team (directly observed treatment)

8.5 Diagnosis of possible *B. pertussis* disease

Possible *B. pertussis* disease in a study volunteer will be a clinical diagnosis made by a study clinician based on early symptoms such as rhinorrhoea, nasal congestion, sore throat, leucocytosis (WBC $\geq 11.5 \times 10^9/l$) and/or CRP >20 mg/l. If there is a suspicion of early *B. pertussis* disease, extra safety bloods may be taken to confirm the findings and a throat swab for respiratory PCR panel and a nasopharyngeal swab for Bp culture will be taken. Treatment will be started immediately if the volunteer has severe symptoms such as a persistent dry cough, a raised temperature (>38.0 °C) or feeling generally unwell. Volunteers with mild symptoms may be observed for up to two days and treatment started if their symptoms are persistent or progressive over two days.

The PI will be informed immediately about any early sign of *B. pertussis* disease (see Figure 9).

Figure 9: Actions to be taken when symptoms of early *B. pertussis* disease are suspected



8.6 Treatment in case of possible *B. pertussis* disease

Once treatment is indicated the procedures that are planned for the day 14 will be done. Azithromycin 500mg orally will be given immediately and continued once a day for a total of 3 days. Volunteers will be kept admitted for 48 hours after azithromycin therapy is started, during which monitoring and sampling will be performed similar to day 15 and 16 (eradication day +1 and +2) to measure clearance of Bp.

It will be emphasised that contact rules will need to be adhered to until the nasopharyngeal culture taken 2 days after eradication therapy (day 16) is negative for *B. pertussis*.

After discharge the volunteers will have an extra follow up visit at 2 weeks and will then be followed up at 4, 8, 26 and 52 weeks.

If two volunteers have confirmed *B. pertussis* disease no other volunteers will be challenged until the data is reviewed by the external safety committee. The study steering committee will review the available data and propose to the external safety committee what inoculum dose and eradication day will be used for the next volunteers. The study can only be continued after approval by the external safety committee.

Confirmed pertussis cases will be reported to Public Health England (PHE) by telephone as soon as is practicable and in writing within 3 days as per local guidelines (19).

Severe illness requiring admission to the intensive care unit

In the unlikely event of volunteers experiencing severe illness during the period of the study, there is a 24/7 intensive care service available at the University Hospital of Southampton NHS Foundation Trust. In case of resuscitation of the volunteer being required, a 24/7 resuscitation service is available in the NIHR Wellcome Trust Clinical Research Facility, which is considered a hospital ward for resuscitation purposes and which is located in the centre of the University Hospital of Southampton.

8.7 Possible complications during the study

8.7.1 Phlebotomy

The maximum volume of blood drawn over the study period, 580 ml, over approximately 12 months should not compromise healthy volunteers. There may be minor bruising, local tenderness or pre-syncope symptoms associated with venepuncture, which will not be documented as AEs if they occur.

8.7.2 Nasal washes, nasopharyngeal swab, throat swab, saliva sample and nasosorption fluid sample

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

8.7.3 Inoculation with *B. pertussis*

Inoculation with *B. pertussis* suspension can cause some irritation of the nasal mucosa that will disappear within a few seconds. Very occasionally, instillation may induce coughing or sneezing, but the risk of this can be reduced by slow instillation down the superior wall of the nares. Transmission to staff is prevented in this event by use of PPE and vaccination of staff. If, in the judgement of the volunteer or research staff, a sneeze is likely to have expelled the inoculum, then the inoculation may be repeated once after waiting a period of 30 minutes.

8.7.4 *B. pertussis* disease

Although the aim of the challenge model is to establish colonisation with *B. pertussis*, disease may occur due to the unknown incubation time of *B. pertussis*. It is expected that if treatment is started early (see paragraph 8.3), there will be a reduced risk of disease and complications in comparison to natural infection where treatment is often delayed due to the typically mild and non-specific presentation.

Reported symptoms of *B. pertussis* disease in adults include coughing 91% (mean duration: 54 days), in 80% this cough lasts ≥ 21 days, whoops (8%), cough followed by vomiting and/or choking (53%) and cough disturbing sleep (52%), sweating attacks (14%), pharyngeal symptoms (37%), influenza-like symptoms (30%), sneezing attacks (22%), hoarseness (18%), sinus pain (16%) and headaches (14%). Adolescents and adults can develop complications from pertussis, but they occur less frequently and are usually less severe than in children. Reported complications of *B. pertussis* disease include urinary incontinence, rib fracture, pneumothorax, inguinal hernia, aspiration, pneumonia, seizures and otitis media (23).

8.7.5 Azithromycin

Azithromycin is used to eradicate *B. pertussis* colonisation at the end of the experimental colonisation period. It is a licenced drug in the UK for the treatment of pertussis, and the treatment consists of a dose of 500 mg once daily for three days.

Azithromycin is generally well tolerated, but occasionally causes some side effects. The side effects include:

- Common: Abdominal discomfort; diarrhoea; nausea; vomiting
- Uncommon: Cholestatic jaundice; hepatotoxicity; rash
- Rare: Antibiotic-associated colitis; arrhythmias; pancreatitis; QT interval prolongation; Stevens-Johnson syndrome; toxic epidermal necrolysis
- Frequency no known: Reversible hearing loss (sometimes with tinnitus) can occur after large doses

Azithromycin does not interfere with the contraceptive pill.

Volunteers should contact the clinical study team urgently if any of these occur.

8.7.6 Withdrawal of a volunteer

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

- Administrative decision by the Investigator.
- Ineligibility (either arising during the study or retrospectively, having been overlooked at screening).
- Significant protocol deviation.
- Volunteer non-compliance with study requirements or infection prevention rules.
- An AE, which requires discontinuation of the study involvement or results in inability to continue to comply with study procedures.
- The external safety committee may recommend withdrawal of volunteers.

The reason for withdrawal from further study procedures will be recorded in the CRF. Other than in the case of complete consent withdrawal, long-term safety data collection, including some procedures, such as safety bloods, will be continued. For all AEs, appropriate follow-up visits or medical care will be arranged, with the agreement of the volunteer, until the AE has resolved, stabilised or a non-trial related causality has been assigned. Any volunteer who withdraws consent or is withdrawn from further study procedures may be replaced.

- If a volunteer withdraws from the study after having had the *B. pertussis* inoculation but before the eradication treatment, an appropriate, curative course of antimicrobial therapy must be completed. The importance of this will be emphasised to volunteers at screening.
- If a volunteer withdraws from the study after having had the *B. pertussis* inoculation, infection prevention measures must be adhered to. The importance of this will be emphasised to volunteers at screening.

- If a volunteer withdraws from the study samples collected before their withdrawal from the trial will be used/stored unless the volunteer specifically requests otherwise. Data from volunteers withdrawn from the study will be included in the analysis of results relating to the study's primary objective.
- In all cases of subject withdrawal, excepting those of complete consent withdrawal, long-term safety data collection will continue as appropriate if subjects have received the inoculum.
- If volunteers withdraw from the study prior to its completion they will be offered financial re-imburement corresponding to the number of visits attended and days admitted.

8.7.7 Non-compliance with study requirements

If the volunteer is not following the instructions of the clinical team and is therefore compromising their own safety and that of the staff or environment, the challenge will be stopped and eradication therapy will be given. This will be reported as an AE. If volunteers are excluded from the study prior to its completion they will be offered financial re-imburement corresponding to the number of visits attended and days admitted.

8.7.8 Missing volunteer

In the unlikely event that a volunteer goes missing and is or uncontactable by telephone after the challenge and before completion of an appropriate course of antimicrobial therapy, the following stakeholders will be informed;

- All investigators
- The volunteer's nominated contact and next of kin
- The trial sponsor
- The hospital trust R&D department
- Public Health England (PHE)

9. Follow up at weeks 4, 8, 26 and 52

At week 4, 8, 26 and 52 after the challenge the volunteer will be reviewed by the clinical team. The study doctor will take a medical history asking if any new medical issues have arisen since the challenge. The health of household contacts will be reviewed and if there are any signs of pertussis in the household, the person is advised to contact their GP. A physical examination will be conducted to check for any abnormalities. Vital signs like heart rate, blood pressure, respiratory rate and temperature will be recorded. Blood samples and nasal samples will be taken at follow up visits (See Table 4).

9.1 Emergency contact

Before the challenge the volunteer must check that his/her telephone is working and ensure that they are contactable. A contact card will be given with the name of the study, the study group and an emergency telephone number (07771 674842) and instructions when to call will be given.

Subjects will be encouraged to contact one of the investigators on the 24 hour emergency mobile study telephone number if they develop symptoms between the regular follow up

1
2
3 visits. The investigator will consider an extra clinical review if the volunteer has any
4 symptoms that are moderate or severe.
5
6

7 8 **10 Laboratory procedures**

9 10 **10.1 Laboratory work**

11 Standard operating procedures for all laboratory work will be followed. Investigators will
12 conform to established laboratory safety standards.
13

14 15 **10.2 Storage of samples**

16 17 **Safety blood samples**

18 Safety blood samples will be labelled with the hospital number and directly sent to the
19 clinical laboratory of University Hospital Southampton for analysis of safety bloods.
20

21 22 **Immunology samples**

23 Samples for further immunological analysis will be labelled with the study specific participant
24 number and time point code. According to the study specific laboratory manual some
25 samples will be processed in the NIHR-WTCRF lab or University of Southampton
26 Laboratory, others will be frozen and stored in the -80°C freezer for later analyses or
27 shipment.
28

29
30 Serology and immunological assays will be analysed at collaborating laboratories of the
31 Periscope consortium. Immunological assays will be conducted according to the procedures
32 established in the test laboratories. Left over samples will be send to the Periscope-bio-
33 bank.
34

35 36 **Microbiology samples and environmental samples**

37 Microbiology samples and environmental samples will be labelled with the study specific
38 participant number and time point code. Cultures will be performed within 48 hours, other
39 samples will be processed, frozen and stored in the -80°C freezer according to the study
40 specific laboratory manual.
41

42 43 **10.3 Labelling of samples**

44 Samples will be clearly identified with the unique identifier and visit number, and recorded in
45 the study sample log.
46
47

48 49 **11. Assessment of safety**

50 Safety of the volunteers will be assessed by analysing the frequency, incidence and nature
51 of adverse events and serious adverse events arising during the study.
52

53 54 **11.1 Definitions**

55 56 **Adverse Event (AE)**

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

1
2
3 associated with the study intervention, whether or not considered related to the study
4 intervention.
5

6 **Adverse Reaction (AR)**

7 An AR is any untoward or unintended response to the inoculum. This means that a causal
8 relationship between the inoculum and an AE is at least a reasonable possibility, i.e., the
9 relationship cannot be ruled out. All cases judged by either the reporting medical investigator
10 or the sponsors as having a reasonable suspected causal relationship to the inoculum (i.e.
11 possibly, probably or definitely related to the inoculum) will qualify as adverse reactions.
12
13

14 **Unexpected Adverse Reaction (UAR)**

15 An adverse reaction, the nature or severity of which is not consistent with the applicable
16 information about the inoculum in the protocol, is considered as an unexpected adverse
17 reaction.
18
19

20 **Serious Adverse Event (SAE)**

21 An SAE is an AE that results in any of the following outcomes, whether or not considered
22 related to the study intervention.
23

- 24 • Death (i.e., results in death from any cause at any time)
- 25 • Life-threatening event (i.e., the volunteer was, in the view of the investigator, at
26 immediate risk of death from the event that occurred). This does not include an AE
27 that, if it occurred in a more serious form, might have caused death.
- 28 • Persistent or significant disability or incapacity (i.e. substantial disruption of one's
29 ability to carry out normal life functions).
- 30 • Hospitalisation other than admission in the NIHR-WTCRF, regardless of length of
31 stay, even if it is a precautionary measure for continued observation. Hospitalisation
32 (including inpatient or outpatient hospitalisation for an elective procedure) for a pre-
33 existing condition that has not worsened unexpectedly does not constitute a serious
34 AE.
- 35 • An important medical event (that may not cause death, be life threatening, or require
36 hospitalisation) that may, based upon appropriate medical judgment, jeopardise the
37 volunteer and/or require medical or surgical intervention to prevent one of the
38 outcomes listed above. Examples of such medical events include allergic reaction
39 requiring intensive treatment in an emergency room or clinic, blood dyscrasias, or
40 convulsions that do not result in inpatient hospitalisation.
- 41 • Congenital anomaly or birth defect.
- 42
- 43
- 44
- 45
- 46
- 47

48 **Serious Adverse Reaction (SAR)**

49 An adverse event (expected or unexpected) that is both serious and, in the opinion of the
50 reporting investigator or sponsors, believed to be possibly, probably or definitely due to the
51 inoculum or any other study treatments, based on the information provided in the protocol.
52
53

54 **Suspected Unexpected Serious Adverse Reactions (SUSARs)**

55 A SUSAR is a SAE that is unexpected and thought to be possibly, probably or definitely
56 related to the inoculum.
57

58 **11.2 Causality assessment**

59 For each AE, an assessment of the relationship of the AE to the study intervention(s) will be
60 undertaken. The relationship of the adverse event with the study procedures will be

categorised as unrelated, unlikely to be related, possibly related, probably related or definitely related (see Table 6). An intervention-related AE refers to an AE for which there is a possible, probable or definite relationship to the study intervention. The investigator will use clinical judgment to determine the relationship. Alternative causes of the AE, such as the natural history of pre-existing medical conditions, concomitant therapy, other risk factors and the temporal relationship of the event to the challenge will be considered and investigated.

Table 6: Guidelines for assessing the relationship of inoculation to an AE

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

11.3 Reporting procedures for AEs

If an adverse event occurs in this research project it will first be reported to the on duty clinical research fellow or research physician, who will investigate and complete an adverse event report form. If the AE is considered to be related and/or serious, a report will be written and sent to the chief and principal investigators (Professor R.C. Read, and Professor S.N. Faust) who will review the report and inform the Chair (or nominated alternate committee member) of the external safety committee. The Sponsor and HRA will be informed if the AE is assessed by the investigators or the external safety committee as having potential to cause harm to the volunteer or subsequent volunteers.

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

11.3.1 Severity grading of clinical and laboratory adverse events

The severity of clinical and laboratory adverse events will be assessed according to the scales in Table 7.

Table 7: Severity grading criterion for AEs.

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

11.3.2 Reporting procedures for serious AEs (SAEs)

In order to comply with current regulations on serious adverse event reporting to regulatory authorities, the event will be documented accurately and notification deadlines respected. SAEs will be reported to the Principal Investigator immediately when the study team is aware of their occurrence, as described in the relevant SOP. The external safety committee will be notified of SAEs deemed possibly, probably or definitely related to study interventions; this will be done immediately (within 24 hours) when the investigators are aware of their occurrence. Copies of reports will be forwarded to the sponsor. SAEs will not normally be reported to the ethical committee(s) unless there is a clinically important increase in occurrence rate, an unexpected outcome, or a new event that is likely to affect safety of trial volunteers, at the discretion of the Chief Investigator and/or external safety committee. In addition to the expedited reporting above, the investigator shall include all SAEs in the annual report for the HRA and sponsor.

11.3.3 Reporting procedures for SUSARs

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

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

11.3.4 Regular safety report

A safety report will be made after the first five volunteers are challenged and this report will be sent to the external safety committee. Permission from external safety committee is needed to continue challenging the next volunteers. After this initial report this safety-report procedure will be repeated after every 5 volunteers.

An annual safety report for the study will be prepared by the anniversary of the first approval date of the study from the regulatory authority. This will be submitted to the sponsor, the external safety committee, the HRA and the scientific advisory group of Periscope.

11.3.5 Procedures to be followed in the event of abnormal findings

Abnormal clinical findings from medical history, examination or blood tests, will be assessed as to their clinical significance using the table in Appendix B. If a test is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains

clinically significant, the volunteer will be informed and appropriate medical care arranged as appropriate with the permission of the volunteer. Decisions to exclude the volunteer from the enrolling in the trial or to withdraw a volunteer from the trial will be at the discretion of the Investigator.

11.3.6 Foreseeable medical occurrences

The following medical occurrences are foreseeable:

- Local abnormal sensation in the nose following inoculation.
- Local bruises from taking blood
- Local expected AEs: rhinorrhoea, nasal congestion, epistaxis, sneezing, ear pain, eye pain, sore throat, cough, dyspnoea
- Systemic expected AEs: Tiredness, headache, pyrexia, feeling generally unwell
- Adverse reactions to azithromycin, as detailed in the Summaries of product characteristics (SmPCs)

11.3.7 Adverse events of special interest

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

- Complications of *B. pertussis* disease including: urinary incontinence, rib fracture, pneumothorax, inguinal hernia, aspiration, pneumonia, seizures and otitis media.
- Severe hypersensitivity reactions to the inoculum (eg. anaphylaxis)
- Overdosing of the inoculum (see paragraph 5.7)
- Confirmed *B. pertussis* infection in a household member

11.4 Safety profile review

The safety profile will be assessed on an on-going basis by the investigators and study committees (see Table 8).

Table 8: An overview of safety committees and reporting activities

	AE	SAE	SUSAR	After first 5 volunteers	After every 10 volunteers	Annual safety report (AE/SAE/SUSAR)
Chief/principal investigator	If related and/or severe: <24 hours	Immediately	Immediately			
Sponsor	If having potential to cause harm: <24 hours	If deemed possibly, probably or definitely related to study interventions: <24 hours	Within required timelines			X
HRA and EAB	If having potential to cause		Within required timelines			X

	harm: <24 hours					
External safety committee	If related and/or severe: <24 hours	If deemed possibly, probably or definitely related to study interventions: <24 hours		X	X	X

11.4.1 Study steering committee

The study steering committee will provide real-time safety oversight on the trial. They will be executing the protocol and responsible for the day to day running of the trial. The study steering committee, chaired by the CI, is responsible for making the safety report and submitting it to the relevant committees

The study steering committee will review SAEs deemed possibly, probably or definitely related to study interventions. The study steering committee has the power to terminate the study if deemed necessary following a study intervention-related SAE.

11.4.2 External Safety Committee

Prior to recruitment we will establish an independent external safety committee (ESC) whose role is to provide overall supervision for the study and provide advice through its independent Chair. The ESC will function as data and safety monitoring board. The ultimate decision for the continuation of the trial lies with the Study Steering Committee following advice from the ESC and the study advisory committee.

The ESC will be responsible for reviewing and assessing this protocol prior to commencement of the trial, interim monitoring of safety and effectiveness, and trial conduct. The ESC will provide a recommendation to the Study Steering Committee concerning the continuation of the study.

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

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

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

11.4.3 Study Advisory Committee

The study advisory committee has been involved in the development of the protocol and will receive an annual report on the progression of the trial. The members of the study advisory

committee will advise the study steering committee on any issues that are encountered during the trial.

11.4.4 Health Research Authority (HRA), Regional Ethical Committee (REC) and Ethical Advisory Group (EAG) Periscope

The protocol and study documents will be approved by the EAG of the Periscope consortium, including an external and independent expert on relevant aspects of ethics, before being reviewed by the HRA and REC. The study will only commence after approval of the HRA and REC. A progress report will be submitted to the HRA 12 months after the date on which the favourable opinion was given. A report by the EAG on the current ethical status and compliance of Periscope will be submitted to the IMI with the Periodic Reports.

11.4.5 Scientific Steering committee Periscope

The protocol and study related documents will be sent for approval to the scientific steering committee of the Periscope consortium prior submission to REC.

11.4.5 Innovative Medicines Initiative (IMI)

As IMI is funding this study through the Periscope consortium, study documentation including the protocol and annual reports will be sent to IMI, as per contract.

11.6 Safety stopping and holding rules

11.6.1 Holding rules

Safety holding rules have been developed for use in the unlikely event of a concern arising regarding the safety of the inoculum for future volunteers

Holding rules:

- Rib fracture, pneumothorax, inguinal hernia, aspiration, pneumonia, seizures and otitis media
- Severe *B. pertussis* disease needing hospitalisation

If the holding rule has been met, subsequent inoculation of volunteers will only start if External Safety Committee, study sponsor and chief investigator agree to it and the following considerations are discussed:

- Relationship of the AE or SAE to the inoculation
- Relationship of the AE or SAE to the inoculation procedure, or other possible causes of the event
- If appropriate, additional screening or laboratory testing which could be provided to other volunteers to identify those who may develop similar symptoms, and necessary alterations to the current Volunteer Information Sheet (VIS)
- New, relevant safety information from ongoing research programmes of the Periscope consortium.

All inoculated subjects will be followed for safety follow up until resolution or stabilisation (if determined to be chronic sequelae) of their AEs.

11.6.2 Individual stopping rules (will apply to all volunteers)

In addition to the above stated group holding rules, stopping rules for individual volunteers will apply (i.e., indications to withdraw individuals from further participation in the study). The

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3 CI and PI will have the responsibility to consider withdrawal from further participation in the
4 study, in consultation with the ESC taking into account the safety of that individual.
5

6
7 In addition to these pre-defined criteria, the study can be put on hold upon advice of the
8 ESC, Chief Investigator, Study Sponsor or Ethical Committee for any single event or
9 combination of multiple events which, in their professional opinion, jeopardise the safety of
10 the subjects or the reliability of the data.
11

12. Analysis and Statistical considerations

12.1 Endpoints of the study

- Safety endpoints:
 - Occurrence of possible or confirmed *B. pertussis* disease.
 - Occurrence of unsolicited adverse events within the study period
 - Occurrence of serious adverse events within the study period
- Where relevant, presence or acquisition of colonisation of *B. pertussis* will be correlated with immunology parameters.

25
26 Safety analysis will be carried out for all volunteers that received the inoculum, regardless of
27 whether or not they complete the study.
28

29 This is an observational and descriptive safety study, where volunteers will be inoculated
30 with *B. pertussis*. Approximately 65 volunteers in total receive the inoculum, approximately
31 35 in phase A, and 30 in phase B. This sample size should allow determination of the
32 magnitude of the outcome measures, especially of serious and severe adverse events,
33 rather than aiming to obtain statistical significance.
34
35

12.2 Sample size calculation

36
37 Immune response to *B. pertussis* will be assessed by a variety of immunological assays. The
38 key assessment in this trial is whether colonisation and seroconversion of anti-PT IgG can
39 be achieved in 70% of the challenged volunteers.
40
41

42 No previous trials have been done using wild type *B. pertussis* in a human challenge model.
43 Previous human challenge studies using nasal inoculation showed the following results:
44

- *N. lactamica* (49) – 35% of the challenged volunteers (n=41) were colonised, mean pre-challenge specific IgG level was 559 IU/mL, mean post challenge specific IgG level was 1100 IU/mL. No significant increase was observed for carriage-negative individuals and the control group.
- *S. pneumoniae* (42) – 10-60% of the challenged volunteers (n=80) were colonised, mean pre-challenge anti-PS IgG was 1312 IU/mL, mean post challenge anti-PS IgG was 2797 IU/mL. No significant increase was observed for carriage-negative individuals
- Genetically modified *B. pertussis* (39) – 58% of the challenged volunteers (n=12) were colonised, mean pre-challenge anti-PT IgG was 6 IU/mL, mean post challenge anti-PT IgG was 25 IU/mL. No significant increase was observed for carriage-negative individuals and the control group.

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2
3 At the above levels of response, using a baseline mean anti-PT IgG of 7 IU/mL, a three-fold
4 improvement in antibody immunogenicity 28 days following the inoculation would represent a
5 biologically important / relevant outcome.
6

7
8 The geometric mean titre is used to evaluate vaccine immunogenicity. A review analysing
9 acellular pertussis vaccines of different companies showed a 95% confidence interval for
10 anti-PT IgG after vaccination of 94.3 (88.8-100.3) ,N=486 and 51.3 (47.9-54.9), N=476. The
11 SD (log10 scale) is $\text{SQRT}(486) \cdot \log(100.3/88.4)/4 = 0.30$ and $\text{SQRT}(476) \cdot \log$
12 $(54.9/47.9)/4 = 0.32$ (50). Using the average of 0.31 for the sample size calculation to detect
13 a 3 fold effect (0.477 on a log10 scale) with 80% power gives N=7 in each group. With 15 in
14 each group a difference of 2.1 fold with 80% power can be detected. Because we expect
15 approximately 50-70% of the SI group will be colonised we need to enrol n = 30 volunteers
16 in the SI group.
17
18

19
20 This sample size calculation might be adjusted based on the results of phase A.
21

22 **12.3 Statistical analysis**

23 Statistical analysis will be performed by Nick Andrews, statistician Public Health England.
24

25 Confidence intervals will be calculated where appropriate and the data will undergo
26 significance testing using contingency table analysis. Categorical variables will be presented
27 as percentages with contingency table analysis including X^2 test and Fisher's exact tests.
28 This analysis will be performed for colonisation data. Serological data will be analysed using
29 1-way analysis of variance. For serological analysis among smaller groups, a Mann-Whitney
30 test will be used. Log-transformed data will be used to construct areas under the curve for
31 comparison of immune responses over the 4-week study period.
32
33
34

35 **13. Study quality and management procedures**

36 **13.1 Protocol and study documents**

37 The protocol and study documents (volunteer information sheet and informed consent form)
38 will be sent to SAB, EAG, IMI, HRA, REC and local R&D before the commencement of the
39 research. HRA and REC approval will be provided to IMI before the commencement of the
40 research. Detailed information will be provided to the IMI on the procedures that will be used
41 for the recruitment of participants (number of participants, inclusion/exclusion criteria,
42 direct/indirect incentives for participation, the risks and benefits for the participants).
43
44
45
46
47
48

49 **13.2 Investigator procedures**

50 Approved site-specific SOPs will be used at all clinical and laboratory sites.
51

52 **13.3 Monitoring**

53 Study monitoring will be provided by the Sponsor according to ICH Good Clinical Practice
54 (GCP). Following written standard operating procedures, the monitors will verify that the
55 clinical trial is conducted and data are generated, documented and reported in compliance
56 with the protocol, GCP and the applicable regulatory requirements. The investigator sites will
57 provide direct access to all trial related source data/documents and reports for the purpose
58 of monitoring and auditing by the sponsor and inspection by local and regulatory authorities.
59
60

13.4 Study Amendments

No amendments to this protocol will be made without consultation with, and agreement of, the Sponsor. Any amendments to the trial that appear necessary during the course of the trial must be discussed with the investigator and sponsor concurrently. If agreement is reached concerning the need for an amendment, it will be produced in writing by the chief investigator and will be made a formal part of the protocol following ethical and regulatory approval (NRES-REC SOPs – Version 5.1 March 2012: http://www.hra.nhs.uk/wp-content/uploads/2013/08/NRES_SOPs_v5.1_2012.03.14.pdf). Any amendments to study documents will follow established HRA and REC requirements.

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

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

The final protocol for phase B will be presented to the REC committee as a substantial protocol amendment, including details of the results from phase A and how these results determined the standard inoculum dose and colonisation period used in Phase B. This substantial amendment will be reviewed at a full REC meeting. Before submission for ethical and regulatory approval it will be reviewed by the scientific advisory board of Periscope, and it will be send to IMI.

13.5 Protocol deviation

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

13.6 Quality control, Quality Assurance and statutory inspections

Steps to be taken to ensure the accuracy and reliability of data include the selection of qualified investigators, review of protocol procedures with the investigator and study-site personnel before the study, periodic monitoring visits by the sponsor, and direct transmission of clinical laboratory data from a central laboratory into the database. Written instructions will be provided for collection, handling, storage, and shipment of samples.

Guidelines for CRF completion will be provided and reviewed with study-site personnel before the start of the study. The sponsor will review CRF for accuracy and completeness during on-site monitoring visits and after transmission to the sponsor; any discrepancies will be resolved with the investigator or designee, as appropriate. After upload of the data into the study database they will be verified for accuracy and consistency with the data sources.

The UHS R&D department staff will provide Quality assurance (QA) and perform internal audits to check that the trial is being conducted, data recorded, analysed and accurately reported according to the protocol, Sponsor's SOPs and in compliance with GCP. The audits will also include laboratory activities according to an agreed audit schedule. The internal

1
2
3 audits will supplement the sponsor's monitoring process and will review processes not
4 covered by the sponsor's monitor.
5

6
7 The Sponsor, trial site and ethical committee may carry out audit to ensure compliance with
8 the protocol, GCP and appropriate regulations. GCP inspections may also be undertaken by
9 the regulatory authority to ensure compliance with protocol and national regulations. The
10 sponsor will assist in any inspections.
11

12 **13.7 Serious breaches**

13
14 A serious breach is defined as "A breach of GCP or the trial protocol which is likely to effect
15 to a significant degree – the safety or physical or mental integrity of the subjects of the trial;
16 or the scientific value of the trial."
17

18
19 In the event that a serious breach is suspected the sponsor will be informed as soon as
20 possible and in turn will notify the HRA and external safety committee within 7 days.
21

22 **13.8 Study Completion/Termination**

23
24 The study is considered completed upon last volunteer/last visit at the site. The end of study
25 is defined as the completion of the testing of samples, to be achieved no later than 12
26 months after the date of the last visit of the last volunteer. The data will be sent to the
27 Periscope consortium and sponsor in the timeframe specified in the Clinical Trial Agreement.
28

29
30 The study steering committee, together with the sponsor and Scientific Advisory Committee,
31 can decide to close the study early if e.g. the safety or physical or mental integrity of the
32 subjects of the trial is likely to be affected by the study or if the scientific value of the trial is
33 affected.
34

35 **13.9 Exploitation and dissemination**

36
37 This study is likely to yield a new model that can be used to evaluate vaccine efficacy and
38 discovery of new biomarkers and as such will be of potential use both to government
39 agencies and to pharmaceutical industry. Reviewing drafts of the manuscripts, abstracts,
40 press releases and any other publications arising from the study will follow the consortium
41 agreement of Periscope. Findings will be published in peer reviewed open access journals
42 as soon as possible, even where results prove negative. The authors will acknowledge that
43 the study is funded by IMI. The results of the study will be disseminated at relevant
44 international scientific meetings. Once this model is established, all future publications
45 arising will acknowledge the funder. Data from the study may also be used as part of a
46 thesis for a PhD or MD.
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50 A lay summary of the results of the study will be sent to volunteers after the trial has been
51 published. An annual report in plain English will be made available on the website of
52 periscope.
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55 **14. Ethics**

56 **14.1 Declaration of Helsinki**

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58 The Investigator will ensure that this study is conducted according to the principles of the
59 current revision of the Declaration of Helsinki 2008.
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14.2 Good clinical practice (GCP) guidelines

The Investigators will ensure that this study is conducted in full conformity to the guidelines for GCP (CPMP/ICH/135/95) July 1996. This Good Clinical Practices document describes the responsibilities and expectations of all participants in the conduct of clinical trials, including investigators, monitors, and sponsors.

14.3 Informed consent

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

14.4 Informing volunteers general practitioners

A letter for the participants' general practitioners describing the study and the participants' involvement will be sent to the general practitioners on the day of the screening visit. This will include contact details for the research team and the contact number for the infectious diseases unit at UHS NHS FT. The GP will be asked to confirm eligibility or send the medical history.

14.5 Research ethics committee

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

14.6 Volunteer confidentiality

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

15. Data handling and record keeping

15.1 Data handling

The chief investigator will be responsible for delegating the receiving, entering, cleaning, querying, analysing and storing of all data that accrues from the study in the site file held in the NIHR-WTCRF. Data handling is specified in the study specific Data Management Plan. The investigators will enter the data into the volunteers' case report file (CRF), which will be

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3 in a paper format. This includes safety data, laboratory data (both clinical and
4 immunological) and outcome data. This process will be quality controlled by a separate staff
5 member. Anonymised data will be transcribed to an electronic database, which will be
6 retained in accordance with the University of Southampton's Research Data Management
7 policy and is shared with a central Periscope database that is accessible to Periscope
8 partners only. Transcription to an electronic database will be checked by a separate study
9 team member, and the monitor. Data processing and the electronic data will be checked
10 regularly by an independent data manager.
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13 **15.2 Record keeping**

14 The investigators will maintain and retain appropriate medical and research records and
15 essential documents for this trial in compliance with ICH E6 GCP and regulatory and
16 institutional requirements for the protection of confidentiality of volunteers. The chief
17 investigator, co-investigators and clinical research nurses will have access to records. The
18 investigators will permit authorised representatives of the sponsor, regulatory agencies and
19 the monitors to examine (and when required by applicable law, to copy) clinical records for
20 the purposes of quality assurance reviews, audits and evaluation of the study safety and
21 progress.
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26 **15.3 Source data and case report forms (CRFs)**

27 All protocol-required information will be collected in CRFs designed by the investigator. All
28 source documents, excluding hospital records, will be filed in the NIHR-WTCRF. Source
29 documents are original documents, data, and records from which the volunteer's CRF data
30 are obtained. For this study these will include, but are not limited to; volunteer consent form,
31 the medical file of the volunteer, blood results, GP response letters, laboratory records and
32 correspondence. In the majority of cases, CRF entries will be considered source data as the
33 CRF will be the site of the original recording (i.e. there is no other written or electronic record
34 of data). In this study this will include, but is not limited to, medical history, medication
35 records, vital signs, physical examination records, urine assessments, blood results, adverse
36 event data and details of study interventions. All source data will be stored securely.
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41 **15.4 Data protection**

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

50 **16.1 Financing**

51 The Periscope project has received funding from the Innovative Medicines Initiative 2 Joint
52 Undertaking under grant agreement No 115910. This Joint Undertaking receives support
53 from the European Union's Horizon 2020 research and innovation programme and EFPIA
54 and BMGF and will be supported by funding for Experimental Medicine by the National
55 Institutes of Health Research through support from the Southampton NIHR Wellcome Trust
56 Clinical Research Facility, the Wessex Comprehensive Research Network (CRN) and the
57 PHE.
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16.2 Insurance

Insurance cover for negligent harm caused within the activities stated in the research protocol, this is provided by the University's Clinical trials insurance.

16.3 Compensation for time

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

- Attending screening and follow-up session and providing samples - £21 total (£15/visit plus additional £6 travel expenses)
- Completed days (24 hours) of admission, at a rate of £200 per day

The maximum individual volunteers will be compensated is £3526 and the minimum £15

If volunteers withdraw from the study prior to its completion they will be offered financial reimbursement corresponding to the number of visits attended and days admitted.

Volunteers will receive the total compensation minus £500 at discharge from the unit, and the remaining £500 at their last visit.

17. Appendices

Appendix A: Laboratory values for exclusion:

The following reference ranges are provided for the purpose of guidance only for investigators during the trial. Results during the trial that fall out with these ranges may not be of clinical significance but should be considered on an individual basis. Abnormal results judged of clinical significance should ordinarily be recorded as adverse events (AEs).

Table 9: laboratory values

Biochemistry	Lower limit	Upper limit
C reactive protein [mg/l]	N/A	7.5
Potassium [mmol/L]	3.5	5.3
Sodium [mmol/L]	133	146
Urea [mmol/L]	N/A	7.8
Creatinine [μ mol/L]	N/A	97
Albumin [g/L]	35	N/A
Total bilirubin [μ mol/L]	N/A	20
ALT [IU/L]	N/A	40
ALP [IU/L]	N/A	130
Haematology		
Haemoglobin [g/L]	Male: 130, Female: 120	Male: 170 Female: 150
White Cell Count [$\times 10^9/L$]	4	11
Neutrophil count [$\times 10^9/L$]	2	7.5
Lymphocyte count [$\times 10^9/L$]	1.5	4.0
Platelet Count [$\times 10^9/L$]	150	400
Serum		
Phase A: anti-PT IgG ELISA IU/mL		>20
Toxicology screening		Positive
Urinanalyses		
Protein		2+ or 0.5-1gm loss/day
Blood		2+ confirmed by 5-10 rbc/hpf
Glucose		1+
Pregnancy test		Positive
Nasopharyngeal swab		
Culture		<i>B. pertussis</i>
ECG		
QTc		≥ 440 ms.

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Appendix B: Immunological essays performed within Periscope

Periscope Task	Assay	Day 0	Day 3	Day 4	Day 7	Day 9	Day 11	Day 14	1 month	6 months	12 months
Task 1.1	Serology AMIA	Y							Y	Y	Y
Task 1.2	Serology ELISA	Y							Y	Y	Y
Task 2-5	Functional Ab. (4 assays)	Y							Y	Y	Y
Transcriptomics	Storage	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Task 6	EuroFlow assays*	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Task 7	Rapid T-cell assay	Y			Y			Y			Y
Task 8	B-cell ELISpot	Y			Y			Y			Y
Task 9	Ag-specific B-cells*	Y		Y	Y	Y	Y	Y	Y	Y	Y
Task 10	IGH-IGL genes*	Y		Y	Y	Y	Y	Y	Y		Y
Task 11	Innate assays*	Y	Y	Y							
Task 12	In-depth T-cell studies*	Y			Y			Y			Y
Biobanking	Serum	Y							Y	Y	Y
	PBMC	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y

18. References

1. England PH. Factsheet Pertussis: Public Health England; 2016 [Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/323000/Factsheet_for_healthcare_professionals.pdf.
2. Crowcroft NS, Stein C, Duclos P, Birmingham M. How best to estimate the global burden of pertussis? *The Lancet Infectious diseases*. 2003;3(7):413-8.
3. Collaborators GMACoD. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet (London, England)*. 2015;385(9963):117-71.
4. Pebody RG, Gay NJ, Giammanco A, Baron S, Schellekens J, Tischer A, et al. The seroepidemiology of *Bordetella pertussis* infection in Western Europe. *Epidemiology and infection*. 2005;133(1):159-71.
5. Barkoff AM, Grondahl-Yli-Hannuksela K, He Q. Seroprevalence studies of pertussis: what have we learned from different immunized populations. *Pathogens and disease*. 2015;73(7).
6. WHO. WHO vaccine-preventable diseases: monitoring system. 2016 global summary 2016 [updated 15/06/2016. Available from: http://apps.who.int/immunization_monitoring/globalsummary/incidences?c=GBR.
7. Jackson DW, Rohani P. Perplexities of pertussis: recent global epidemiological trends and their potential causes. *Epidemiology and infection*. 2014;142(4):672-84.
8. Althouse BM, Scarpino SV. Asymptomatic transmission and the resurgence of *Bordetella pertussis*. *BMC medicine*. 2015;13:146.
9. Linnemann CC, Jr., Bass JW, Smith MH. The carrier state in pertussis. *American journal of epidemiology*. 1968;88(3):422-7.
10. Zhang Q, Yin Z, Li Y, Luo H, Shao Z, Gao Y, et al. Prevalence of asymptomatic *Bordetella pertussis* and *Bordetella parapertussis* infections among school children in China as determined by pooled real-time PCR: a cross-sectional study. *Scandinavian journal of infectious diseases*. 2014;46(4):280-7.
11. ClinicalTrials.gov. *Bordetella Pertussis Carriage in College-aged Students*: U.S. National Institutes of Health; 2015 [Available from: <https://clinicaltrials.gov/ct2/show/NCT02215564>.
12. Mertsola J, Ruuskanen O, Eerola E, Viljanen MK. Intrafamilial spread of pertussis. *The Journal of pediatrics*. 1983;103(3):359-63.
13. de Greeff SC, Mooi FR, Westerhof A, Verbakel JM, Peeters MF, Heuvelman CJ, et al. Pertussis disease burden in the household: how to

- 1
2
3
4 protect young infants. *Clinical infectious diseases : an official publication*
5 of the Infectious Diseases Society of America. 2010;50(10):1339-45.
- 6 14. Aintablian N, Walpita P, Sawyer MH. Detection of Bordetella
7 pertussis and respiratory syncytial virus in air samples from hospital
8 rooms. *Infection control and hospital epidemiology*. 1998;19(12):918-23.
- 9 15. Warfel JM, Beren J, Merkel TJ. Airborne transmission of Bordetella
10 pertussis. *The Journal of infectious diseases*. 2012;206(6):902-6.
- 11 16. Kretzschmar M, Teunis PF, Pebody RG. Incidence and
12 reproduction numbers of pertussis: estimates from serological and social
13 contact data in five European countries. *PLoS medicine*.
14 2010;7(6):e1000291.
- 15 17. MacDonald H, MacDonald EJ. Experimental pertussis. *The Journal*
16 of infectious diseases. 1933;53(3):328-30.
- 17 18. Vink MA, Bootsma MC, Wallinga J. Serial intervals of respiratory
18 infectious diseases: a systematic review and analysis. *American journal*
19 of epidemiology. 2014;180(9):865-75.
- 20 19. England PH. Guidelines for the Public Health Management of
21 Pertussis in England London 2016 [Available from:
22 https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/541694/Guidelines_for_the_Public_Health_Management_of_Pertussis_in_England.pdf.
23
24
25
26
27
28
29
30
31
32
- 33 20. Huygen K, Rodeghiero C, Govaerts D, Leroux-Roels I, Melin P,
34 Reynders M, et al. Bordetella pertussis seroprevalence in Belgian adults
35 aged 20-39 years, 2012. *Epidemiology and infection*. 2014;142(4):724-8.
- 36 21. He H, Yao P, Zhou Y, Deng X, Pan J. Is Pertussis Infection
37 Neglected in China? Evidence from a Seroepidemiology Survey in
38 Zhejiang, an Eastern Province of China. *PloS one*.
39 2016;11(5):e0155965.
- 40 22. Deville JG, Cherry JD, Christenson PD, Pineda E, Leach CT,
41 Kuhls TL, et al. Frequency of unrecognized Bordetella pertussis
42 infections in adults. *Clinical infectious diseases : an official publication of*
43 the Infectious Diseases Society of America. 1995;21(3):639-42.
- 44 23. Rothstein E, Edwards K. Health burden of pertussis in adolescents
45 and adults. *The Pediatric infectious disease journal*. 2005;24(5
46 Suppl):S44-7.
- 47 24. Farizo KM, Cochi SL, Zell ER, Brink EW, Wassilak SG, Patriarca
48 PA. Epidemiological features of pertussis in the United States, 1980-
49 1989. *Clinical infectious diseases : an official publication of the Infectious*
50 *Diseases Society of America*. 1992;14(3):708-19.
- 51 25. De Serres G, Shadmani R, Duval B, Boulianne N, Dery P, Douville
52 Fradet M, et al. Morbidity of pertussis in adolescents and adults. *The*
53 *Journal of infectious diseases*. 2000;182(1):174-9.
- 54
55
56
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46
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50
51
52
53
54
55
56
57
58
59
60
26. Kretsinger K, Broder KR, Cortese MM, Joyce MP, Ortega-Sanchez I, Lee GM, et al. Preventing tetanus, diphtheria, and pertussis among adults: use of tetanus toxoid, reduced diphtheria toxoid and acellular pertussis vaccine recommendations of the Advisory Committee on Immunization Practices (ACIP) and recommendation of ACIP, supported by the Healthcare Infection Control Practices Advisory Committee (HICPAC), for use of Tdap among health-care personnel. *MMWR Recommendations and reports : Morbidity and mortality weekly report Recommendations and reports / Centers for Disease Control.* 2006;55(Rr-17):1-37.
 27. Kilgore PE, Salim AM, Zervos MJ, Schmitt HJ. Pertussis: Microbiology, Disease, Treatment, and Prevention. *Clinical microbiology reviews.* 2016;29(3):449-86.
 28. Altunaiji S, Kukuruzovic R, Curtis N, Massie J. Antibiotics for whooping cough (pertussis). *The Cochrane database of systematic reviews.* 2007(3):CD004404.
 29. Pichichero ME, Hoeger WJ, Casey JR. Azithromycin for the treatment of pertussis. *The Pediatric infectious disease journal.* 2003;22(9):847-9.
 30. Tiwari T, Murphy TV, Moran J. Recommended antimicrobial agents for the treatment and postexposure prophylaxis of pertussis: 2005 CDC Guidelines. *MMWR Recommendations and reports : Morbidity and mortality weekly report Recommendations and reports / Centers for Disease Control.* 2005;54(Rr-14):1-16.
 31. Parnham MJ, Erakovic Haber V, Giamarellos-Bourboulis EJ, Perletti G, Verleden GM, Vos R. Azithromycin: mechanisms of action and their relevance for clinical applications. *Pharmacology & therapeutics.* 2014;143(2):225-45.
 32. Mooi FR, van der Maas NAT, de Melker HE. Pertussis resurgence: waning immunity and pathogen adaptation – two sides of the same coin. *Epidemiology & Infection.* 2014;142(04):685-94.
 33. Klein NP, Bartlett J, Rowhani-Rahbar A, Fireman B, Baxter R. Waning protection after fifth dose of acellular pertussis vaccine in children. *The New England journal of medicine.* 2012;367(11):1012-9.
 34. Warfel JM, Papin JF, Wolf RF, Zimmerman LI, Merkel TJ. Maternal and neonatal vaccination protects newborn baboons from pertussis infection. *The Journal of infectious diseases.* 2014;210(4):604-10.
 35. Higgs R, Higgins SC, Ross PJ, Mills KH. Immunity to the respiratory pathogen *Bordetella pertussis*. *Mucosal immunology.* 2012;5(5):485-500.
 36. Guiso N, Njamkepo E, Vie le Sage F, Zepp F, Meyer CU, Abitbol V, et al. Long-term humoral and cell-mediated immunity after acellular pertussis vaccination compares favourably with whole-cell vaccines 6

- 1
2
3
4 years after booster vaccination in the second year of life. *Vaccine*.
5 2007;25(8):1390-7.
- 6 37. Elahi S, Holmstrom J, Gerdt V. The benefits of using diverse
7 animal models for studying pertussis. *Trends in microbiology*.
8 2007;15(10):462-8.
- 9 38. Merkel TJ, Halperin SA. Nonhuman primate and human challenge
10 models of pertussis. *The Journal of infectious diseases*. 2014;209 Suppl
11 1:S20-3.
- 12 39. Thorstensson R, Trollfors B, Al-Tawil N, Jahnmatz M, Bergstrom J,
13 Ljungman M, et al. A phase I clinical study of a live attenuated *Bordetella*
14 *pertussis* vaccine--BPZE1; a single centre, double-blind, placebo-
15 controlled, dose-escalating study of BPZE1 given intranasally to healthy
16 adult male volunteers. *PloS one*. 2014;9(1):e83449.
- 17 40. Evans CM, Pratt CB, Matheson M, Vaughan TE, Findlow J, Borrow
18 R, et al. Nasopharyngeal colonization by *Neisseria lactamica* and
19 induction of protective immunity against *Neisseria meningitidis*. *Clinical*
20 *infectious diseases : an official publication of the Infectious Diseases*
21 *Society of America*. 2011;52(1):70-7.
- 22 41. A D, al. e. Carriage of the commensal *Neisseria lactamica* inhibits
23 acquisition and carriage of *Neisseria meningitidis* in the nasopharynx. .
24 Association of Physicians Annual Meeting, Belfast 2013. 2013.
- 25 42. Ferreira DM, Neill DR, Bangert M, Gritzfeld JF, Green N, Wright
26 AK, et al. Controlled human infection and rechallenge with
27 *Streptococcus pneumoniae* reveals the protective efficacy of carriage in
28 healthy adults. *American journal of respiratory and critical care medicine*.
29 2013;187(8):855-64.
- 30 43. Sciences AoM. Microbial Challenge Studies of Human Volunteers.
31 2005.
- 32 44. R M. Microbial Challenge Studies of Human Volunteers. . The
33 Academy of Medical Sciences. 2005.
- 34 45. Agency HP. HPA guidelines for the Public Health Management of
35 Pertussis in Healthcare Settings 2012 [updated 25/10/2012. Available
36 from:
37 https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/323103/HPA_Guidelines_for_the_Public_Health_Management_of_Pertussis_Incidents_in_Healthcare_Settings_PB6401-Oct_2012.pdf.
38
- 39 46. Naclerio RM, Meier HL, Kagey-Sobotka A, Adkinson NF, Jr.,
40 Meyers DA, Norman PS, et al. Mediator release after nasal airway
41 challenge with allergen. *The American review of respiratory disease*.
42 1983;128(4):597-602.
- 43 47. Gritzfeld JF, Roberts P, Roche L, El Batrawy S, Gordon SB.
44 Comparison between nasopharyngeal swab and nasal wash, using
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3 culture and PCR, in the detection of potential respiratory pathogens.
4 BMC research notes. 2011;4:122.

5
6 48. England PH. Guidance Whooping cough (pertussis): immunisation
7 of healthcare workers 2013 [Available from:

8
9 [https://www.gov.uk/guidance/whooping-cough-pertussis-immunisation-](https://www.gov.uk/guidance/whooping-cough-pertussis-immunisation-of-healthcare-workers)
10 [of-healthcare-workers](https://www.gov.uk/guidance/whooping-cough-pertussis-immunisation-of-healthcare-workers).

11
12 49. A D, al. e. Carriage of the commensal *Neisseria lactamica* inhibits
13 acquisition and carriage of *Neisseria meningitidis* in the nasopharynx.
14 Association of Physicians Annual Meeting, Belfast 2013. 2013.

15
16 50. Edwards KM, Berbers GA. Immune responses to pertussis
17 vaccines and disease. *The Journal of infectious diseases*. 2014;209
18 Suppl 1:S10-5.
19
20
21
22
23
24
25
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BMJ Open

Investigating *Bordetella pertussis* colonisation and immunity: protocol for an inpatient controlled human infection model.

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2017-018594.R1
Article Type:	Protocol
Date Submitted by the Author:	28-Aug-2017
Complete List of Authors:	de Graaf, Hans; University Hospital Southampton NHS Foundation Trust, NIHR Clinical Research Facility; University of Southampton , Faculty of Medicine Gbesemete, Diane; University Hospital Southampton NHS Foundation Trust, NIHR Clinical Research Facility; University of Southampton , Faculty of Medicine Gorringe, Andrew; Public Health England Porton Diavatopoulos, Dimitri; Radboud University Medical Centre, Radboud Centre for Infectious Diseases, Laboratory of Pediatric Infectious Diseases Kester, Kent; Sanofi Pasteur Inc Faust, Saul; University of Southampton, UK, NIHR Wellcome Trust Clinical Research Facility Read, Rob; University of Southampton , Faculty of Medicine; Institute of Life Science, NIHR Biomedical Research Centre
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Immunology (including allergy), Public health, Respiratory medicine, Infectious diseases
Keywords:	<i>Bordetella pertussis</i> , Human Challenge Study, Whooping Cough, Colonisation, Vaccine development

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3 **Investigating *Bordetella pertussis* colonisation and immunity: protocol for an inpatient controlled**
4 **human infection model.**
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47 **Key words:** *Bordetella pertussis*, human challenge study, whooping cough, colonisation, Vaccine
48 Development
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54 **Abstract:**

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56 INTRODUCTION: We summarise an ethically approved protocol for the development of an
57 experimental *Bordetella pertussis* human challenge colonisation model. Globally *B. pertussis* is one
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3 of the leading causes of vaccine preventable death. Many countries have replaced whole cell
4 vaccines with acellular vaccines over the last 20 years during which pertussis appears to be resurgent
5 in a number of countries in the developed world that boast high immunization coverage. The
6 acellular vaccine provides relatively short-lived immunity and, in contrast to whole cell vaccines, may
7 be less effective against colonisation and subsequent transmission. To improve vaccine strategies, a
8 greater understanding of human *B. pertussis* colonisation is required.
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13 METHODS AND ANALYSIS: A controlled human colonisation model will be developed over two
14 phases. In phase A, a low dose of the *B. pertussis* inoculum will be given intranasally to healthy
15 participants. This dose will be escalated or de-escalated until colonisation is achieved in
16 approximately 70% (95% confidence interval: 47-93%) of the exposed volunteers without causing
17 disease. The colonisation period, shedding and exploratory immunology will be assessed during a 17
18 day inpatient stay and follow-up over one year. The dose of inoculum which achieves 70%
19 colonisation will then be confirmed in phase B, comparing healthy participants exposed to *B.*
20 *pertussis* with a control group receiving a sham inoculum.
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27 ETHICS AND DISSEMINATION: This study has been approved by the ethical committee reference:
28 17/SC/0006, 24 February 2017. Findings will be published in peer reviewed open access journals as
29 soon as possible.
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36 **Strengths and limitations of this study:**

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40 - Close in-patient observation after *B. pertussis* exposure allows close monitoring of volunteer
41 safety, prevents transmission to people at risk of pertussis disease and provides the opportunity to
42 assess shedding of *B. pertussis*.
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45 - The proposed dose escalation schedule is designed to optimise volunteer safety.
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48 - As part of the Periscope Consortium it will be possible to compare the immunology and
49 microbiology results of this human challenge study with vaccine induced immunity and results of
50 animal challenge studies.- The individual variation and the low number of participants might
51 influence the external validity of the results
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55 - Aiming for 70% colonisation may be suboptimal for future vaccine efficacy studies using this model.
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Introduction:

Pertussis, also called whooping cough, is an acute bacterial infection caused by *Bordetella pertussis* (Bp), an exclusively human pathogen. Although it can affect people of all ages, young unimmunised infants are the most vulnerable group with the highest rates of complications and death (1). In 1999 there were an estimated 48.5 million pertussis cases in children worldwide and 295,000 deaths (2), but based on sero-epidemiological prevalence studies the number of asymptomatic Bp infections may be much higher (3). By 2013, mortality fell to about 60,600 (interquartile range 22,300-136,800) children per year, still making it one of the leading causes of vaccine-preventable death (4).

Pertussis vaccines have been included in National Immunisation Programmes since their introduction in the 1940s-1950s. Acellular pertussis vaccines (aP) have a favourable reactogenicity profile in comparison to whole-cell pertussis vaccines (wP) and are currently mostly used in industrialised countries to immunise against pertussis (5). However, many countries in the developed world that boast high immunization coverage have seen an increase in the incidence of pertussis over the past 20 years (6). Five main hypotheses have been proposed to contribute to this resurgence: 1) rapid waning of immunity following vaccination, especially with aP (7), 2) the very different immune response profile induced by aP compared to wP vaccines (8), 3) adaptation of Bp to escape protective immunity, 4) low vaccine coverage and 5) less effective reduction of transmission from infected individuals vaccinated with aPs (9). The latter is supported by baboon studies that have demonstrated that vaccination with aP prevents severe disease but does not prevent asymptomatic infection, i.e. colonisation (10). Studies in mice are consistent with these findings and demonstrate that protective immunity is more effective and persistent when induced by natural infection or wP than by aP (11). To study the pathogenesis of pertussis a variety of animal models have been used, including mice, rabbits, guinea pigs, and newborn piglets (12). However, there are still important knowledge gaps relating to human immunity to Bp and it is not clear to what extent these observations in animal models translate into clinical practice. This paucity of knowledge hampers the development of improved vaccines and the design of better vaccination strategies against pertussis in infants, adolescents and adults.

The deliberate infection of human volunteers with micro-organisms has contributed uniquely to our understanding of the pathogenesis, immune responses and the treatment and prevention of numerous microbial diseases including pneumococcal disease (13), influenza, cholera, typhoid and hepatitis (14). We aim to develop a safe human challenge **colonisation** model to allow a more thorough understanding of the immune response against wild type Bp and to facilitate development of bioassays and next-generation pertussis vaccines.

Methods:

This is a controlled human infection study consisting of two phases; phase A: development of a Bp human challenge colonisation model and phase B: Development of a modified Bp human challenge colonisation model in which we will compare participants receiving the standard inoculum (SI) with a control group receiving a sham inoculum. This article summarises the protocol for phase A.

Phase A will be a dose finding phase in which the dose of inoculum will be escalated or de-escalated to find the Standard Inoculum (SI), defined as the dose of Bp that will safely cause colonisation in approximately 70% (95% confidence interval: 47-93%) of the exposed participants. Colonisation will be defined as a positive culture of Bp from the nasopharyngeal swab specimen between day 0 and 14. After five participants have received the inoculum, colonisation will be assessed and the following five participants will receive either the same dose or an escalated or decreased dose (Figure 1). Once a dose of inoculum achieves a colonisation rate of 70% then that dose will be used to inoculate further participants until a total of 10 participants have been colonised. This will require inoculation of approximately 14 participants with that dose, which will then be defined as the standard inoculum dose.

The various samples (nasopharyngeal swab, throat swab, nasal wash, and nasal fluid sample) will be compared to identify the technique that yields the highest sensitivity of Bp detection to inform the design of Phase B. The seroconversion rate will be measured comparing pre- and post-inoculation serum samples of participants infected after receiving the standard inoculum. Seroconversion will be defined as a 3-fold rise in anti-PT IgG titre (IU/mL) from day 0 to day 28. We will then identify the 'colonisation period' which is defined as the earliest day after inoculation on which colonisation of the nasopharynx (as detected by the most sensitive technique) is observed in 100% of those volunteers who subsequently seroconvert at day 28. The colonisation period will be used in the protocol for Phase B to minimise the duration of infection prior to eradication of Bp by antibiotics.

Study objectives:

The primary objective of phase A of this study is to determine the dose of the standard inoculum in participants who do not have evidence of recent Bp exposure. The standard inoculum is defined as the dose of Bp causing colonisation in approximately 70% of the participants between day 0 and 14 without causing Bp disease after being challenged with this dose at day 0. An endpoint of 70% is used to avoid, if possible, a 'saturating dose' that results in non-physiological colonisation by Bp in participants, but still induces sufficiently high colonisation rates to enable potential future vaccine

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3 efficacy trials. Secondary endpoints will explore the ability to dose the inoculum accurately, the pre-
4 and post-challenge Bp-specific immunity in healthy participants and the environmental shedding of
5 Bp following nasal inoculation (see table 1). Feedback and recommendations from Patient and Public
6 Involvement (PPI), in addition to participant questionnaires from previous malaria human challenge
7 studies performed in Southampton, have been incorporated into this study.
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10 11 *Challenge strain*

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14 The Bp isolate to be used in this human colonisation model is B1917, which is representative of
15 current isolates in Europe (15, 16). The strain, isolated in 2000 from a Dutch patient with Bp disease,
16 is characterised as *ptxP3-ptxA1-prn2-fim3-2, fim2-1* MLVA27, PFGE BpSR11 and expresses pertactin
17 (PRN), pertussis toxin (PT) and filamentous hemagglutinin (FHA). This strain has been extensively
18 characterised in the mouse model as well as by proteomics and transcriptomics and has a closed
19 genome available (16). It is fully sensitive to azithromycin *in vitro*.
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24 The inoculum has been prepared by Q Biologicals (Ghent, Belgium) according to good manufacturing
25 practice (GMP) standard in licensed GMP facilities and using a process free of animal-derived
26 products. The identity and purity of the cell bank have been confirmed, in addition to any other
27 quality specifications agreed within the consortium and needed for compliance with regulatory
28 requirements. Before intranasal inoculation, there will be no further culture of the challenge
29 inoculum at the clinical site. The dose and purity of the inoculum will be determined after
30 inoculation for quality assessment.
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36 37 *Study setting*

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39 This is one of several other clinical and preclinical studies of the Periscope consortium
40 (<http://periscope-project.eu/consortium/>) which brings together internationally renowned scientists
41 with many years of experience in Bp research, clinical trials, bioinformatics, immunology and public
42 health. The aim of the consortium is to promote scientific and technological innovation in pertussis
43 vaccine development and to foster the creation of a laboratory and scientific network that facilitates
44 the testing and helps expedite the development of novel pertussis vaccines in Europe.
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50 The study will be conducted by the University of Southampton in the National Health Institute for
51 Health Research Clinical Research Facility (CRF) Southampton.
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53 54 *Recruitment*

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56 Participants will be recruited via the following:
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- Southampton CRF and Periscope websites – information about the study will be available on the website with a downloadable volunteer information sheet
- Posters in public places, including buses and trains, university campus, student bars, halls of residence, health centres etc. with the agreement of the owner / proprietor
- Newspapers or other literature for circulation
- Press release
- Clinical Research Update magazine
- A post on a Twitter, Facebook or Gumtree account owned and operated by our group
- Email distribution to individuals who have already expressed an interest in taking part in any clinical trial at the CRF Southampton.
- Southampton CRF Database of Healthy Volunteers: individuals from this database have previously expressed an interest in receiving information about future studies for which they may be eligible.

A recruitment and volunteer management plan has been formulated to prioritise and co-ordinate these strategies. Potential volunteers who are interested will be sent the volunteer information sheet and will be invited for a screening visit. During this visit they will be given an opportunity to discuss the study questions and complete a pre-consent questionnaire to ensure they understand the study. The informed consent procedure includes specific infection prevention information regarding the measures that are taken to prevent transmission during admission to the research unit. Once consent has been given their eligibility will be assessed. This will include a general health questionnaire, which is a screening tool to identify common psychiatric conditions. The medical history will be checked with the general practitioner. Participants will be offered reimbursement for their time, travel and inconvenience.

Eligibility criteria

Healthy volunteers, men and women, aged 18-45 without known or suspected recent pertussis infection (anti-PT IgG >20 IU/mL) will be recruited for phase A. Care will be taken not to recruit from vulnerable groups (mental health or other impaired capacity issues). Specific inclusion and exclusion criteria can be found in the online supplementary Table 1. Female volunteers are required to use an effective form of contraception for the duration of their participation in this study. These criteria should minimise the risk of complicated disease or transmission of Bp to risk groups and aim to select a homogenous group that will be able to adhere to the scheduled admission and follow up visits.

Interventions

The start of volunteer participation is defined as the screening visit. The end of volunteer participation is defined as the last visit. The duration of involvement in this study from screening will be approximately 56 weeks. A detailed schedule of the interventions is shown in table 2.

A week before the challenge nasal samples will be collected (see figure 2). On the day of the challenge the participant will be admitted to a designated area in the CRF and will have access to a dedicated individual bedroom, shared toilet, shower and recreational areas during their stay in the facility. Participants will be required to wear a surgical mask covering their nose and mouth when outside their personal room, e.g. in the recreational room, unless outside in open air. Participants will be allowed to leave the CRF for a maximum of two hours twice a day, during the daytime. When outside the CRF, participants will be asked to adhere to infection prevention rules based on Public Health England guidelines. These include avoiding contact with people at risk of pertussis, avoiding direct face to face contact and wearing a surgical face mask when inside. When they leave or come back to the designated area, they will be escorted by a member of the study team. The Bp inoculum will be prepared and administered following study-specific standard operating procedures (SOPs) which are based on previous human challenge studies using nasal inoculation with *N. lactamica* (17) (18). One vial of inoculum will be removed from the freezer, thawed and diluted to the required inoculum dose. The participant will be positioned supine with neck extended, mouth open, and breathing normally through their mouth. 0.5 mL of the inoculum will be gently expelled into each nostril, while the participant is positioned with mouth open, neck extended and breathing normally through their mouth. After the inoculum is administered the vial residuum will be diluted and cultured for five days on *Bordetella* selective medium for determination and viable counts of Bp and on non-selective medium to assess purity of the inoculum.

During admission, clinical observations and symptoms of possible early pertussis disease such as rhinorrhoea, nasal congestion, epistaxis, sneezing, ear pain, eye pain, sore throat, cough, dyspnoea, feeling generally unwell, tiredness and headache will be reviewed six times per day. Regular nasal, mouth, throat and blood samples will be taken to assess the microbial dynamics and the host response to the challenge. Environmental samples will be taken to assess shedding. These include cultures and quantitative polymerase chain reaction (qPCR) of: face masks, contact areas in the personal room, air sampling of the personal room and during aerosol producing activities. Nasopharyngeal samples will be requested from CRF staff members with participant contact to monitor transmission of viable *B. pertussis*.

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3 In Phase A, azithromycin 500 mg will be given once a day for three days to eradicate possible Bp
4 colonisation on day 14 or to treat possible early stage Bp disease before day 14 following national
5 guidelines (19). After start of the treatment, participants will remain in the hospital for another 48
6 hours to assess eradication success and environmental shedding after eradication.
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10 A nasopharyngeal swab will be taken prior to discharge which will be 48 hours after the start of
11 eradication therapy. If this sample is positive for B. pertussis within five days, the course of
12 azithromycin will be repeated. Follow up visits will take place at week 4, 8, 26 and 52.
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16 If, following inoculation, the participant develops symptoms of early pertussis disease, azithromycin
17 will be commenced according to Public Health England treatment guidelines. This will be done
18 following a pre-defined treatment algorithm (see Figure 3) which is designed to exclude trivial viral
19 infection or transient and non-specific upper respiratory tract symptoms. After treatment the
20 participant will continue to be included in the study and specimens will be collected as if they had
21 received eradication treatment at day 14 per protocol.
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25 26 *Safety*

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28 Our priority is to develop this model without causing harm to the individual or the environment. An
29 important facet of a safe model will be to limit participant exposure to Bp to the minimum dose and
30 number of days required to signal successful colonisation. Safety considerations in the protocol
31 include:
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36 1. The dose of the inoculum will start low and will be modified to the lowest dose required to
37 effectively establish colonisation with regular reviews of the available safety data by the
38 study steering committee and the external safety committee.
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40 2. The timing of eradication will be modified to optimise the safety of the participants by
41 exposing them to the minimal effective period of colonisation.
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43 3. The incubation time for the catarrhal phase is unknown, but is estimated to be 7 – 22 days.
44 After inoculation of the participants, there will therefore be a moderate risk of symptom
45 development because eradication therapy will initially be given at day 14.
- 46
47 4. If a participant shows signs of possible disease there will be a low threshold to eradicate
48 colonisation.
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50 5. Bp disease in adults is often atypical and relatively mild. Treatment is not thought to reduce
51 the duration of coughing in natural infection, but adults with natural infection will not
52 usually receive treatment until a late stage of the disease, if at all. Early treatment (as
53 proposed in this study) is considered more likely to reduce symptoms quickly
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- 3 6. The likelihood of severe disease is extremely low and the risk of harm to the participants,
- 4 staff and others can be minimised by admitting the participants as inpatients in the
- 5 Southampton CRF which is a NIHR facility specifically funded to conduct higher risk
- 6 experimental medicine in a safe National Health Service (NHS) environment. Household
- 7 contacts will not be asked to provide informed consent, but volunteers with household
- 8 members within the risk groups for pertussis disease will be excluded from the study.
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- 13 7. All health care workers involved in this study will be vaccinated against Bp at least two
- 14 weeks before working with participants that have received the inoculum unless they have
- 15 been vaccinated against Bp in the last five years (20).
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18 An external safety committee of independent infectious disease experts will assess the safety of
19 study after every five participants and advise on the continuation of the study.
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21 *Sample size*

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24 Phase A is a dose finding study and the sample size has been estimated with the assumption that we
25 will colonise no, or only a few, participants at the initial low dose, allowing for escalation of the dose
26 based on colonisation frequency and safety parameters. We are aiming to achieve colonisation and
27 seroconversion in 10 participants with the standard inoculum dose of Bp.
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31 *Statistical analysis*

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34 Formal statistical comparisons of colonisation/ seroconversion will not be carried out in phase A. In
35 this dose finding phase, decisions to escalate or de-escalate the dose will be based on the results
36 accumulating over the progression of the study.
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40 *Ethics and dissemination:*

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43 In the UK and EU, an experimental human challenge study with a wild-type bacterium falls outside
44 the European Clinical Trials Directive (14). Participants in challenge trials are healthy volunteers who
45 do not obtain direct health benefit from participation. Potential volunteers will be informed of all
46 conceivable risks and have adequate time to decide on their individual participation in relation to
47 the risks involved and financial compensation for the time and inconvenience of taking part. Medical
48 ethicists have argued that it is a healthy adult's right to self-determine their participation in such
49 trials in relation to the risk (Controlled Human Infection Studies in the development of Vaccines &
50 Therapeutics Wellcome Trust Scientific Conference, January 9-11, 2013, University of Cambridge,
51 UK).
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3 The protocol has been reviewed by independent peer reviewers including experts from Public Health
4 England, and worldwide experts on pertussis within the Periscope consortium ([www.periscope-](http://www.periscope-project.eu)
5 [project.eu](http://www.periscope-project.eu)) who have assessed the safety and the quality of the study, the study design and the
6 feasibility of the study objectives. The protocol has been reviewed and approved by the South
7 Central - Oxford A Research Ethics Committee (REC reference: 17/SC/0006, 24 February 2017) and
8 the UK Health Research Authority (IRAS project ID: 219496, 1/3/2017). Findings will be published in
9 peer reviewed open access journals as soon as possible. The final protocol for phase B will be
10 presented as a substantial protocol amendment, because it will be based on the standard inoculum
11 and colonisation period identified in phase A as well as the outcome of the exploratory immune
12 assays performed during this phase.
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21 Discussion

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24 To the best of our knowledge this will be the first adult human challenge study with wild type Bp. A
25 paediatric Bp human challenge study was performed in 1933 in which two naive and two vaccinated
26 children were exposed to 140 cfu of intranasal Bp (21). While the vaccinated children did not
27 develop whooping cough the naive children developed severe disease including anorexia, fever,
28 severe paroxysmal cough, leucocytosis, lymphocytosis and positive cough plate cultures. They
29 received no treatment and improved by day 35. This study confirmed that pertussis disease is
30 caused by the bacillus of Bordet and Gengou and could be prevented by the experimental Bp vaccine
31 that was used.
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38 In a recent first in human study, healthy volunteers were given a live attenuated Bp strain as a nasal
39 vaccine (22). The inoculated strain was genetically modified; dermonecrotic toxin and tracheal
40 cytotoxin were removed, and PT was genetically detoxified by two independent mutations,
41 removing the toxic activity of PT without affecting its immunogenic properties. Three groups, each of
42 12 participants, were inoculated with 10^3 , 10^5 or 10^7 colony forming units respectively. Colonisation
43 was seen in one subject in the low dose, one in the medium dose and five in the high dose group.
44 Adverse events occurred in similar frequency in all groups, including the placebo group and were
45 found to be trivial. The effect of the genetic changes on the ability to colonise are unknown but it is
46 expected to have a significant effect on colonisation.
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53 The inoculum required to colonise previously vaccinated adults exposed to natural infection with Bp
54 is unknown, but is assumed to be higher than that used in the paediatric challenge study because
55 previously exposed and vaccinated adults are included. The initial inoculum dose used in our study
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3 (1000 cfu) has been chosen as a dose low enough to be assumed to be safe, but high enough to
4 allow accurate monitoring.
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7 This model presumes that Bp disease is preceded by colonisation. Direct evidence for this has proven
8 hard to obtain in surveillance studies (23, 24). Potential reasons for this are the use of inadequate
9 sampling methods or insufficiently sensitive assays, or that colonisation is a very short phenomenon
10 and often missed in a cross-sectional study. Seroprevalence studies do show there is a natural
11 boosting of anti-PT IgG levels, suggesting asymptomatic infection/colonisation is quite frequent (25-
12 27).
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17 Results of two previous studies demonstrated statistically significant correlations between
18 protection against pertussis disease and the presence of anti-PT IgG in pre-exposure sera (28, 29).
19 Because of this we will exclude volunteers with a high anti-PT IgG in phase A, although there is no
20 evidence high anti-PT IgG levels correlate with protection against colonisation. In phase B this will
21 not be an exclusion criterion and the correlation between anti-PT IgG levels and protection against
22 colonisation will be assessed.
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28 To minimise the risk for the participant this controlled infection study is not a disease model, as was
29 the case with many other challenge studies (30) (31), but a colonisation model. The risk of symptom
30 development in Phase A is moderate rather than low because eradication therapy is not planned to
31 be given until day 14 to allow an adequate immune response to develop. Eradication therapy will be
32 given earlier if symptoms develop that are suggestive of pertussis disease. This approach is
33 considered to be acceptable because the risk of severe disease in healthy adults, who are previously
34 vaccinated and probably naturally exposed to Bp, is extremely low (32, 33). Antibiotic treatment is
35 known to be effective at eradicating Bp if given early after diagnosis, but does not alter the
36 subsequent clinical course of the illness (34), especially if administered beyond 2–3 weeks after the
37 onset of symptoms. In this study treatment will be given at an early stage of the infection, which we
38 predict will have a more positive effect on the disease course.
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46 We have implemented comprehensive transmission prevention measures, including an in-patient
47 stay of 17 days. The shedding of Bp, the colonisation frequency and colonisation period will be
48 evaluated during phase A alongside the host immune response, to inform a more directed approach
49 in phase B.
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53 The target colonisation frequency of 70% was chosen partly to be appropriate for future studies
54 requiring a comparison of colonised participants with non-colonised/protected participants, for
55 example studies aiming to identify protective biomarkers. This colonisation frequency might not be
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3 appropriate for studies designed to assess the efficacy of novel vaccines in prevention of
4 colonisation where a near 100% colonisation frequency would be preferable. Therefore the standard
5 inoculum dose may be increased in future studies, depending on the results of this initial study.
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9 We recognise that this method of intranasal inoculation to induce colonisation / infection differs
10 from the natural course of infection. However, this model will provide the opportunity to study the
11 systemic and local immune response to exposure to Bp in a way that would not be feasible in natural
12 human exposure or accurately represented by an animal model of exposure. Because of the atypical
13 and often late presentation of pertussis in adults, knowledge about the features of pre-symptomatic
14 and early infection is lacking. This study will give a unique insight in the initial interaction between
15 bacteria and host during the first two weeks after initial exposure. The development of a safe human
16 challenge model of pertussis, in conjunction with the recently developed baboon model of pertussis,
17 has the potential to provide a path forward for answering critical questions about pertussis
18 pathogenesis and host responses and will likely aid in the development of next-generation pertussis
19 vaccines (35). Within the Periscope consortium we aim to compare the results of this study with
20 animal studies, vaccination studies with acellular and whole cell vaccines and natural infection
21 studies with Bp.
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47
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Full references

1. Public Health England. Factsheet Pertussis: Public Health England; 2016 [Available from: https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/323000/Factsheet_for_healthcare_professionals.pdf.
2. Crowcroft NS, Stein C, Duclos P, Birmingham M. How best to estimate the global burden of pertussis? *The Lancet Infectious diseases*. 2003;3(7):413-8.
3. de Greeff SC, Mooi FR, Westerhof A, Verbakel JM, Peeters MF, Heuvelman CJ, et al. Pertussis disease burden in the household: how to protect young infants. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2010;50(10):1339-45.
4. GBD 2013 Mortality and Causes of Death Collaborators. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. *Lancet (London, England)*. 2015;385(9963):117-71.
5. Zhang L, Prietsch SO, Axelsson I, Halperin SA. Acellular vaccines for preventing whooping cough in children. *The Cochrane database of systematic reviews*. 2014(9):Cd001478.
6. Jackson DW, Rohani P. Perplexities of pertussis: recent global epidemiological trends and their potential causes. *Epidemiology and infection*. 2014;142(4):672-84.
7. Klein NP, Bartlett J, Rowhani-Rahbar A, Fireman B, Baxter R. Waning protection after fifth dose of acellular pertussis vaccine in children. *The New England journal of medicine*. 2012;367(11):1012-9.
8. Brummelman J, Wilk MM, Han WGH, van Els CACM, Mills KHG. Roads to the development of improved pertussis vaccines paved by immunology. *Pathogens and disease*. 2015;73(8):ftv067.
9. Warfel JM, Zimmerman LI, Merkel TJ. Acellular pertussis vaccines protect against disease but fail to prevent infection and transmission in a nonhuman primate model. *Proceedings of the National Academy of Sciences of the United States of America*. 2014;111(2):787-92.
10. Warfel JM, Papin JF, Wolf RF, Zimmerman LI, Merkel TJ. Maternal and neonatal vaccination protects newborn baboons from pertussis infection. *The Journal of infectious diseases*. 2014;210(4):604-10.
11. Higgs R, Higgins SC, Ross PJ, Mills KH. Immunity to the respiratory pathogen *Bordetella pertussis*. *Mucosal immunology*. 2012;5(5):485-500.
12. Elahi S, Holmstrom J, Gerds V. The benefits of using diverse animal models for studying pertussis. *Trends in microbiology*. 2007;15(10):462-8.
13. Ferreira DM, Jambo KC, Gordon SB. Experimental human pneumococcal carriage models for vaccine research. *Trends in microbiology*. 2011;19(9):464-70.
14. The Academy of Medical Science. *Microbial Challenge Studies of Human Volunteers*. 2005.
15. Bart MJ, Zeddeman A, van der Heide HG, Heuvelman K, van Gent M, Mooi FR. Complete Genome Sequences of *Bordetella pertussis* Isolates B1917 and B1920, Representing Two Predominant Global Lineages. *Genome announcements*. 2014;2(6).
16. Bart MJ, van der Heide HG, Zeddeman A, Heuvelman K, van Gent M, Mooi FR. Complete Genome Sequences of 11 *Bordetella pertussis* Strains Representing the Pandemic ptxP3 Lineage. *Genome announcements*. 2015;3(6).
17. Deasy A, Guccione E, Dale A, Andrews N, Evans C, Bennett J, et al. Carriage of the commensal *Neisseria lactamica* inhibits acquisition and carriage of *Neisseria meningitidis* in the nasopharynx. *Association of Physicians Annual Meeting, Belfast 2013*. 2013.
18. Evans CM, Pratt CB, Matheson M, Vaughan TE, Findlow J, Borrow R, et al. Nasopharyngeal colonization by *Neisseria lactamica* and induction of protective immunity against *Neisseria meningitidis*. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America*. 2011;52(1):70-7.
19. Public Health England. *Guidelines for the Public Health Management of Pertussis in England London2016* [Available from:

1
2
3 https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/541694/Guidelines_for_the_Public_Health_Management_of_Pertussis_in_England.pdf.

- 4
5 20. Public Health England. Guidance Whooping cough (pertussis): immunisation of healthcare
6 workers 2013 [Available from: [https://www.gov.uk/guidance/whooping-cough-pertussis-](https://www.gov.uk/guidance/whooping-cough-pertussis-immunisation-of-healthcare-workers)
7 [immunisation-of-healthcare-workers](https://www.gov.uk/guidance/whooping-cough-pertussis-immunisation-of-healthcare-workers).
8
9 21. MacDonald H, MacDonald EJ. Experimental pertussis. *The Journal of infectious diseases*.
10 1933;53(3):328-30.
11 22. Thorstensson R, Trollfors B, Al-Tawil N, Jahnmatz M, Bergstrom J, Ljungman M, et al. A phase
12 I clinical study of a live attenuated *Bordetella pertussis* vaccine--BPZE1; a single centre, double-blind,
13 placebo-controlled, dose-escalating study of BPZE1 given intranasally to healthy adult male
14 volunteers. *PloS One*. 2014;9(1):e83449.
15 23. Linnemann CC Jr., Bass JW, Smith MH. The carrier state in pertussis. *American journal of*
16 *epidemiology*. 1968;88(3):422-7.
17 24. Zhang Q, Yin Z, Li Y, Luo H, Shao Z, Gao Y, et al. Prevalence of asymptomatic *Bordetella*
18 *pertussis* and *Bordetella parapertussis* infections among school children in China as determined by
19 pooled real-time PCR: a cross-sectional study. *Scandinavian journal of infectious diseases*.
20 2014;46(4):280-7.
21 25. Mertsola J, Ruuskanen O, Eerola E, Viljanen MK. Intrafamilial spread of pertussis. *The Journal*
22 *of pediatrics*. 1983;103(3):359-63.
23 26. Huygen K, Rodeghiero C, Govaerts D, Leroux-Roels I, Melin P, Reynders M, et al. *Bordetella*
24 *pertussis* seroprevalence in Belgian adults aged 20-39 years, 2012. *Epidemiology and infection*.
25 2014;142(4):724-8.
26 27. He H, Yao P, Zhou Y, Deng X, Pan J. Is Pertussis Infection Neglected in China? Evidence from a
27 Seroepidemiology Survey in Zhejiang, an Eastern Province of China. *PloS one*. 2016;11(5):e0155965.
28 28. Storsaeter J, Hallander HO, Gustafsson L, Olin P. Levels of anti-pertussis antibodies related to
29 protection after household exposure to *Bordetella pertussis*. *Vaccine*. 1998;16(20):1907-16.
30 29. Cherry JD, Gornbein J, Heininger U, Stehr K. A search for serologic correlates of immunity to
31 *Bordetella pertussis* cough illnesses. *Vaccine*. 1998;16(20):1901-6.
32 30. McCullagh D, Dobinson HC, Darton T, Campbell D, Jones C, Snape M, et al. Understanding
33 paratyphoid infection: study protocol for the development of a human model of *Salmonella enterica*
34 serovar Paratyphi A challenge in healthy adult volunteers. *BMJ open*. 2015;5(6):e007481.
35 31. Rampling T, Ewer KJ, Bowyer G, Bliss CM, Edwards NJ, Wright D, et al. Safety and High Level
36 Efficacy of the Combination Malaria Vaccine Regimen of RTS,S/AS01B With Chimpanzee Adenovirus
37 63 and Modified Vaccinia Ankara Vectored Vaccines Expressing ME-TRAP. *The Journal of infectious*
38 *diseases*. 2016;214(5):772-81.
39 32. Farizo KM, Cochi SL, Zell ER, Brink EW, Wassilak SG, Patriarca PA. Epidemiological features of
40 pertussis in the United States, 1980-1989. *Clinical infectious diseases : an official publication of the*
41 *Infectious Diseases Society of America*. 1992;14(3):708-19.
42 33. De Serres G, Shadmani R, Duval B, Boulianne N, Dery P, Douville Fradet M, et al. Morbidity of
43 pertussis in adolescents and adults. *The Journal of infectious diseases*. 2000;182(1):174-9.
44 34. Altunajji S, Kukuruzovic R, Curtis N, Massie J. Antibiotics for whooping cough (pertussis). *The*
45 *Cochrane database of systematic reviews*. 2007(3):CD004404.
46 35. Merkel TJ, Halperin SA. Nonhuman primate and human challenge models of pertussis. *The*
47 *Journal of infectious diseases*. 2014;209 Suppl 1:S20-3.
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3 *Authors' contributions:*

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5 H. de Graaf, has written the protocol

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8 D. Gbesemete, assisted and advised writing the protocol

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10 Andrew Gorringe, assisted and advised writing the protocol

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12 Dimitri Diavatopoulos, assisted and advised writing the protocol

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14
15 Kent Kester, assisted and advised writing the protocol

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17
18 Saul N. Faust, assisted and advised writing the protocol

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20 Robert C. Read, has written the protocol

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36 *Competing interests statement:*

37
38 Hans de Graaf has received sponsorship from Abbvie to attend a clinical paediatric rheumatology
39 course

40
41
42 Saul N. Faust acts on behalf of the University of Southampton/University Hospital Southampton NHS
43 Foundation trust as chief and principal investigator for clinical trials Sponsored by vaccine and
44 antimicrobial manufacturers but receives no personal payments for the work.
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Table 1: Objectives and endpoints of the study

	Objectives	Endpoints
1A	To determine the dose of the standard inoculum in participants who do not have evidence of recent Bp exposure - Safety	Safety endpoints: - Occurrence of possible or confirmed Bp disease within the study period - Occurrence of unsolicited adverse events within the study period - Occurrence of serious adverse events within the study period
1B	To determine the dose of the standard inoculum in participants who do not have evidence of recent Bp exposure – 70% colonisation	Microbiologically proven Bp colonisation by positive culture of Bp from a nasopharyngeal swab taken between time points day 0 and 14 after being challenged on day 0
2	To evaluate accuracy of the inoculum dosing	Estimation of the actual challenge dose in comparison to the prescribed challenge dose by viable count (cfu/mL) of residual inoculum following inoculation of each participant
3	To describe the human physiological response to Bp challenge in those developing or not developing infection	Description of the clinical course after challenge using, for example: Clinical and laboratory observations such as temperature (°C), systolic blood pressure (mm Hg), diastolic blood pressure (mm Hg), heart rate (beats/min), respiratory rate (breaths/min), O ₂ saturation in blood (%), CRP (mg/L), WBC (10 ⁹ /L), Lymphocyte count (10 ⁹ /L) at various time-points
4	To determine the colonisation period: the earliest day after inoculation at which colonisation of the nasopharynx (as detected by culture) is observed in	A 3-fold rise in anti-PT IgG titre (IU/mL) from day 0 to day 28 will be used as a marker of seroconversion. Colonisation will be detected by positive culture of Bp from a

	100% of those participants who subsequently seroconvert at day 28	nasopharyngeal swab taken between time-points day 0 and 14
5	To determine the characteristics of bacterial dynamics after challenge	Microbiological assays to detect and characterise Bp dynamics after challenge in nasopharyngeal swabs (culture, qPCR and microbiome analyses), nasal wash (culture including semi-quantitative method using cfu count/mL, and precision quantification with qPCR) and sequencing of isolates at various time-points
6	To assess environmental shedding of Bp following nasal inoculation of healthy participants with Bp.	Daily microbiological assays from day 0 to 16 to detect Bp on surface contact (culture and PCR), air sampling (PCR) fingertip culture (culture Bp and PCR), cough box (culture Bp, particle size during various activities: talking, coughing, singing)
7	To determine the eradication frequency of Bp after a three day course of Azithromycin	Microbiological assays after eradication in nasopharyngeal swabs (culture, qPCR and microbiome analyses), nasal wash (culture including semi-quantitative method using cfu count/mL, qPCR) on day 15 and 16
8	To describe the human immune response to challenge, including innate, humoral, cell-mediated and mucosal responses.	Immunological assays to measure innate, humoral, cell-mediated and mucosal responses to challenge in blood (anti-PT IgG (IU/mL), anti-FHA IgG (IU/mL), anti-PRN IgG (IU/mL), anti-FIM IgG (IU/mL), nasal washes (T-cell/B-cell analyses), nasal fluid samples (cytokines) and saliva (Bp specific IgA (IU/mL)) samples, comparing day 0 with day 1, 3, 4, 7, 9, 11, 14, and week 4, 8, 26 and 52.

Bp: *B. pertussis*, cfu: colony-forming units, CRP: C-reactive protein, WBC: white blood cell count, PT: pertussis toxin, PCR: polymerase chain reaction, qPCR: quantitative PCR, IU: international units, PRN: pertactin, FHA: filamentous hemagglutinin, FIM: fimbriae

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Table 2: Scheduled events and interventions:

Day	-30	-7	0	1	2	3	4	5	7	9	11	14	15	16	28	56	183	365
Visit	x	x													x	x	x	x
Admission																		
Inoculum			x															
Bloods	x		x	x	x	x	x		x	x	x	x			x	x	x	x
Urine	x		x															
ECG	x																	
Nasal wash		x					x		x	x	x	x		x	x	x	x	x
Nasal fluid sample		2x				2x	2x	2x	2x	2x	2x	2x	2x	2x	2x	2x	2x	2x
Nasopharyngeal swab	x	x				x		x	x	x	x	x	x	x	x			
Throat swab		x				x		x	x	x	x	x	x	x				
Saliva		x				x		x	x	x	x	x	x	x				
Antibiotic therapy												x	x	x				

ECG: Electrocardiogram

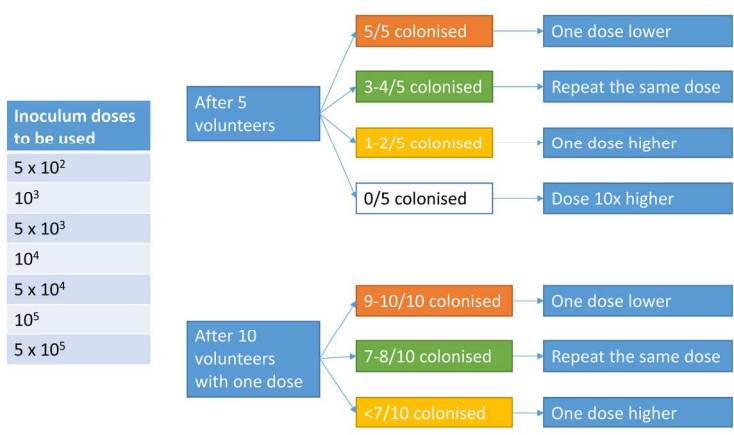
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3 **Figure 1:** Escalating or de-escalating the dose of the inoculum according to colonisation frequency
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5 **Figure 2:** Visits and admission design
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10 **Figure 3:** Actions to be taken when symptoms of early *B. pertussis* disease are suspected
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12 PI: Principal investigator, WBC: White blood cell count, CRP: C-reactive protein, PCR: polymerase
13 chain reaction, URTI: Upper respiratory tract infection
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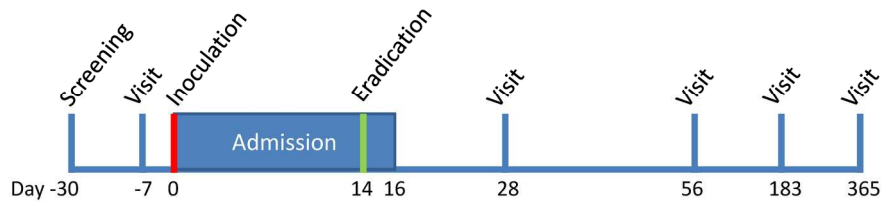
To continue until 10 volunteers are colonised with the same dose of inoculum

Escalating or de-escalating the dose of the inoculum according to colonisation frequency

190x107mm (300 x 300 DPI)

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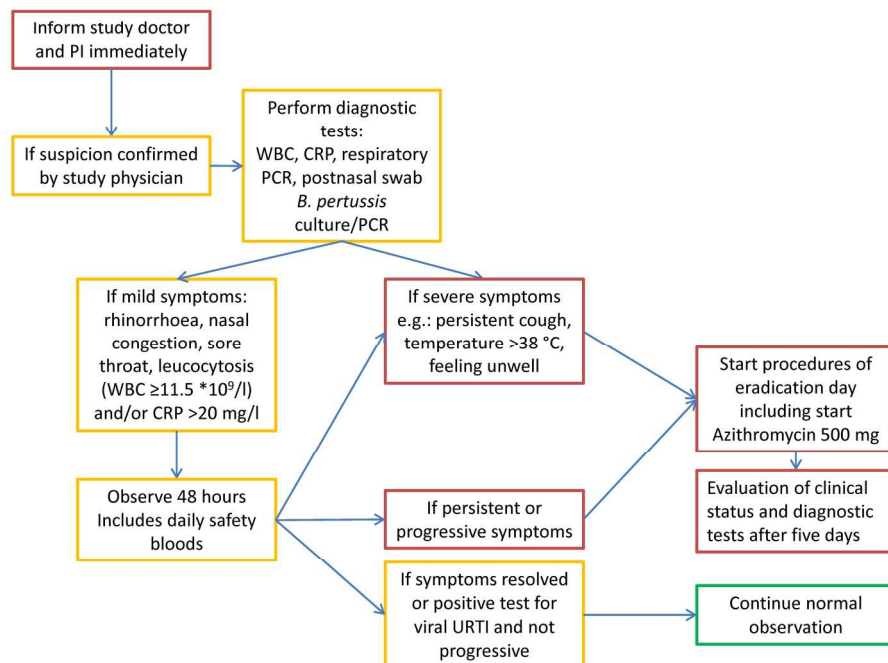
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Visits and admission design
190x142mm (300 x 300 DPI)

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Actions to be taken when symptoms of early *B. pertussis* disease are suspected



Actions to be taken when symptoms of early *B. pertussis* disease are suspected. PI: Principal investigator, WBC: White blood cell count, CRP: C-reactive protein, PCR: polymerase chain reaction, URTI: Upper respiratory tract infection

190x142mm (300 x 300 DPI)

Supplement table 1:

Inclusion and exclusion criteria for the *B. pertussis* human challenge colonisation study

Inclusion criteria	
1	Healthy adults aged 18 to 45 years inclusive at screening
2	Fully conversant in the English language
3	Able to communicate easily by both mobile telephone and text messaging
4	Able and willing (in the investigator's opinion) to comply with all study requirements
5	Written informed consent to participate in the trial
6	Willingness to take an antibiotic regimen after inoculation with <i>B. pertussis</i> according to the study protocol
7	Agreement to be admitted to the NIHR-CRF Southampton for 17 days for phase A (from inoculation until two days after the eradication therapy is given) and for the duration necessary for phase B, depending on phase A results
8	Agree to the infection prevention rules as stated in the volunteer information sheet
9	TOPS registration completed and no conflict found

NIHR-CRF: National Health Institute for Health Research-Clinical Research Facility, TOPS: The Over-volunteering Prevention System

Exclusion criteria	
1.	Individuals who have inviolable commitments within 3 months of discharge from the inpatient phase of the study to make contact with: a. unimmunised or partially immunised children and infants aged < 1 year b. pregnant women >32 weeks who have not received pertussis vaccination at least a v contact
2.	Individuals who have household contacts working with a. unimmunised or partially immunised children and infants aged < 1 year b. pregnant women >32 weeks who have not received pertussis vaccination at least a v contact
3.	Phase A only: Volunteers will be excluded from this study if they have evidence of recent <i>B. pertussis</i> infection, as determined by anti-PT IgG ELISA (>20 IU/mL)
4.	<i>B. pertussis</i> detected on nasopharyngeal swab taken before the challenge
5.	Individuals who have a signs of a current infection at the time of inoculation with <i>B. pertussis</i>
6.	Individuals who have participated in other interventional clinical trials in the last 12 weeks
7.	Individuals who have a history of receiving <i>B. pertussis</i> vaccination in the last 5 years
8.	Individuals who have a history of never being vaccinated against <i>B. pertussis</i>
9.	Current smokers defined as having had a cigarette/cigar in the last week.
10.	Use of systemic antibiotics within 30 days of or during the challenge
11.	Any confirmed or suspected immunosuppressive or immune-deficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) immunosuppressant medication within the past 6 months (topical steroids are allowed)
12.	Use of immunoglobulins or blood products within 3 months prior to enrolment
13.	History of allergic disease or reactions likely to be exacerbated by any component of the inoculum
14.	Contraindications to the use of azithromycin or macrolides
15.	Pregnancy, lactation or intention to become pregnant during the study

1 2 3 4 5 6 7 8	16. Any clinically significant abnormal finding on biochemistry, haematology, toxicology or serological blood tests, urinalysis or clinical examination - in the event of abnormal test results, confirmatory repeat tests will be requested
9 10 11 12 13 14 15	17. Any other significant disease, disorder, or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data, for example recent surgery to the nasopharynx

16 PT: pertussis toxin, IgG: immunoglobulin-G, ELISA: enzyme-linked immunosorbent assay, IU:
17 international units

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4 *Effective contraception for female participants*
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6 Female participants are required to use an effective form of contraception during this study.
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8 Acceptable forms of contraception include:
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- 10 - Established use of oral, injected or implanted hormonal methods of contraception
11 - Placement of an intrauterine device or intrauterine system
12 - Total abdominal hysterectomy
13 - Barrier methods of contraception (condom or occlusive cap with spermicide)
14 - Male sterilisation if the vasectomised partner is the sole partner for the subject
15 - True abstinence when this is in line with the preferred and usual lifestyle of the subject
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