A phase I safety, immunogenicity and dose escalation study of the candidate pan-Sarbeco Coronavirus vaccine pEVAC-PS in SARS-CoV-2 immunised UK healthy adult volunteers

Short Study Title	Phase I Vaccine Study of pEVAC-PS
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Chief Investigator (Clinical):	Professor Saul Faust Director NIHR Southampton Clinical Research Facility University of Southampton C Level, West Wing, Mailpoint 218 University Hospital Southampton NHS Foundation Trust Tremona Road, Southampton, SO16 6YD Tel: 02381 204989 Fax: 02381 205023 Email: s.faust@soton.ac.uk
Co-Investigator (Non-Clinical):	Professor Jonathan Heeney Head, Lab of Viral Zoonotics University of Cambridge, Madingley Road, Cambridge, CB3-0ES Tel: 01223 766250 Email: jlh66@cam.ac.uk
Sponsor Representative:	Mrs Sharon Davies-Dear R&D department Level E, Laboratory & Pathology Block SCBR - Mailpoint 138 University Hospital Southampton NHS Foundation Trust Tremona Road, Southampton, SO16 6YD Tel: 02381 205664 Email: sponsor@uhs.nhs.uk

Site Principal Investigator:	Professor Saul Faust Director NIHR Southampton Clinical Research Facility University of Southampton C Level, West Wing, Mailpoint 218 University Hospital Southampton NHS Foundation Trust Tremona Road, Southampton, SO16 6YD Tel: 02381 204989 Fax: 02381 205023 Email: s.faust@soton.ac.uk
Site Investigators:	Dr Alasdair Munro NIHR Southampton Clinical Research Facility University of Southampton C Level, West Wing, Mailpoint 218 University Hospital Southampton NHS Foundation Trust Tremona Road, Southampton, SO16 6YD Tel: 02381 204989 Fax: 02381 205023 Email: A.Munro@soton.ac.uk
Trial Site:	NIHR Southampton Clinical Research Facility University Hospital Southampton NHS Foundation Trust C Level, West Wing, Mailpoint 218 Tremona Road, Southampton, SO16 6YD
Statistician:	Dr Graham Wheeler Deputy Head of Statistics Imperial Clinical Trials Unit, Imperial College London 1st Floor, Stadium House 68 Wood Lane, London W12 7RH Email: graham.wheeler@imperial.ac.uk
Local Safety Monitor (Chairman of Local Safety Committee):	Professor Robert C. Read University of Southampton C Level, South Laboratory & Pathology Block University Hospital Southampton NHS Foundation Trust Tremona Road, Southampton, SO16 6YD Tel: 02381 206455 Email: r.c.read@soton.ac.uk

Investigational Medicinal Product (IMP) Supplier:	Symbiosis Pharmaceutical Serviced Limited Scion House, Stirling University, Innovation Park Stirling FK9 4NF United Kingdom
External Contract Research Organisation (CRO):	PHARMExcel Ltd Albany Chambers 26 Bridge Road East Welwyn Garden City Hertfordshire AL7 1HL United Kingdom

CRO Project Manager:	Matthew Wilkins Albany Chambers 26 Bridge Road East Welwyn Garden City Hertfordshire AL7 1HL United Kingdom Tel: 0203 642 6654 Email: matthew.wilkins@pharmexcel-cro.com
Site Study Manager	Zoe Swash NIHR Southampton Clinical Research Facility University Hospital Southampton NHS Foundation Trust C Level, West Wing, Mailpoint 218 Tremona Road, Southampton, SO16 6YD Tel: 0238 120 4989 Email: zoe.swash@uhs.nhs.uk

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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 Saul N. Faust. 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 Saul N. Faust.

SIGNATURE PAGE

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Sponsor Study Ref:	RHM MED1776
Protocol Version/Date	v1.0/17th September 2021

The undersigned confirm that the following protocol has been agreed and accepted and that the Chief Investigator agrees to conduct the trial in compliance with the approved protocol and Investigators Brochure and will adhere to the principles outlined in the Medicines for Human Use (Clinical Trials) Regulations 2004 (SI 2004/1031), amended regulations (SI 2006/1928) and any subsequent amendments of the clinical trial regulations, GCP guidelines, the Sponsor's (and any other relevant) SOPs, and other regulatory requirements as amended.
I agree to ensure that the confidential information contained in this document will not be used for any other purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of the Sponsor.

I also confirm that I will make the findings of the trial publicly available through publication or other dissemination tools without any unnecessary delay and that an honest accurate and transparent account of the trial will be given; and that any discrepancies and serious breaches of GCP from the trial as planned in this protocol will be explained.

For and on behalf of the Trial Sponsor:

Signature: Date: ...../...../.....

.....

Name (please print):

.....

Position: .....

Chief Investigator:

Signature: ..... Date: ...../...../.....

Name: (please print):

.....

Contents

Synopsis

Title	A phase I safety, immunogenicity and dose escalation study of the candidate pan-Sarbeco Coronavirus vaccine pEVAC-PS in SARS-CoV-2 immunised UK healthy adult volunteers
Sponsor	University Hospital Southampton NHS Foundation Trust
Trial Centres	*Southampton* - NIHR Clinical Research Facility, Southampton University Hospital NHS Foundation Trust, Southampton, SO16 6YD
Sponsor Study Reference	RHM MED1776
Design	Open label, single-centre, first-in-human, adaptive dose escalation Phase I COVID-19 DNA vaccine trial.
Clinical Phase	I
Population	Healthy, males and non-pregnant females aged 18-50 years who are immunised no less than 12 weeks prior with 2 doses of SARS-CoV-2 vaccine and have no history of serological evidence of prior SARS-CoV-2 infection (seronegative for N)
Sample size	**Total: 36 volunteers** **Stage 1** Group 1: 6 volunteers; 2 doses of pEVAC-PS at 0.2mg (lowest dose) intradermally **Stage 2** subject to safety and immunogenicity analysis from stage 1 after 4w post 2nd dose **Primary immunogenicity target met** Expanded group 1: 12 volunteers; 2 doses of pEVAC-PS at 0.2mg intradermally Review for plan of reduced dose formulation and testing if continued safety and immunogenicity targets met in expanded group **Primary immunogenicity target not met** Group 2a: 9 volunteers; 2 doses of pEVAC-PS at 0.4mg intradermally PLUS Group 2b: 9 volunteers; 2 doses of pEVAC-PS at 0.8mg intradermally **Very Poor/No response** Group 2a: 9 volunteers; 2 doses of pEVAC-PS at 0.8mg intradermally PLUS Group 2b: 9 volunteers; 2 doses of pEVAC-PS at 1.2mg intradermally **Stage 3** subject to safety and immunogenicity analysis from stage 2 after 4w post 2nd dose

	Primary immunogenicity target met Group 3: 12 volunteers, 2 doses of pEVAC-PS at the dose with the best immunogenicity and reactogenicity profile from Groups 2a or 2b **Primary immunogenicity target not met at 2x 0.8mg ID** Group 2c: 12 volunteers; 2 doses of pEVAC-PS at 1.2mg intradermally
Follow up duration	COVID-19 vaccinated volunteers (immunised no less than 12 weeks prior to enrolment), and who have no prior history or serological evidence of SARS-CoV-2 infection will be followed up for a total of approximately 6 months from the time of receiving the first dose of pEVAC-PS (this is an estimate and may vary in accordance with the specified time windows for each attendance).
Planned Trial Period	18 months (this includes up to 6 months during the recruitment and dosing phase with a follow-up period of up to 12 months).
Primary Objective	To assess the safety and reactogenicity of a new pan-Sarbeco Coronavirus DNA candidate vaccine pEVAC-PS in a boosting regime in healthy volunteers.
Secondary Objectives	To assess the humoral immunogenicity of pEVAC-PS when administered to healthy volunteers, through the analysis of SARS-CoV and SARS-CoV-2 RBD antibody titres. Assess optimum dosing of candidate vaccine pEVAC-PS To assess long term (up to 12 months) humoral immunogenicity of the candidate vaccine pEVAC-PS administered by the needle-free technology (PharmaJet)
Investigational Product	pEVAC-PS
Form	Liquid
Dose	pEVAC-PS; 1.2mg ID, 0.8mg ID, 0.4mg ID, 0.2mg ID
Route	Intradermal (ID) administration into the deltoid region of the arm (split left and right with >0.2mg)

Abbreviations

AE	Adverse Event
AR	Adverse Reaction
ALS	Advanced life support
AUC	Area Under the Curve
CI	Chief Investigator
CBF	Clinical Bio-manufacturing Facility
COVID-19	Coronavirus Disease 2019
CRF	Case Report File
CTRG	Clinical Trials Research Governance
DSUR	Development Safety Update Report
EPSC	Early Phase Safety Committee
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immunospot
FIH	First in Human
FBC	Full blood count
GCP	Good Clinical Practice
GMP	Good Manufacturing Practice
GP	General Practitioner
HB	Haemoglobin
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HPA	Health Protection Agency
HRA	Health Research Authority
IB	Investigators Brochure
ICH	International Conference on Harmonisation
ICU	Intensive Care Unit
IDT	Indefinite Delivery type
ILS	Immediate Life Support
IMI	Innovative Medicines Initiative
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
ISF	Investigator Site File

LSC	Local safety committee
LSM	Local safety monitor
MHRA	Medicines and Healthcare Products Regulatory Agency
NHS	National Health Service
NIH	National Institute of Health (USA)
NIHR-CRF	National Institute for Health Research Clinical Research Facility
PBMC	Peripheral blood mononuclear cells
PCR	Polymerase Chain Reaction
LVZ	Laboratory of Viral Zoonotics
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
QP	Qualified Person
REC	Research Ethics Committee
SAB	Scientific Advisory Board
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SARS-CoV or SARS-CoV-1	Severe Acute Respiratory Syndrome Coronavirus
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SDV	Source Data Verification
SOP	Standard Operating Procedure
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
ULN	Upper limit of normal
UK	United Kingdom
VIS	Volunteer Information Sheet
WBC	White blood cells
WHO	World Health Organisation

1. Background & Rationale

1.1 Background

A number of SARS-CoV-2 viruses have been designated as Variants of Concern (VOC) by the WHO because of changes in the spike protein which may contribute to future vaccine escape. Furthermore, there is the constant threat of new and re-emerging (i.e. SARS or SARS-related) Sarbeco (ACE2 using) Coronaviruses.

Since 1966 there have been 7 documented animal to human spillovers of Coronaviruses, 3 of which have caused High Consequence Infectious Diseases in Humans. SARS-CoV caused international epidemics in 2002/2003, and SARS-CoV-2 is the cause of the current global COVID-19 pandemic. These highly contagious, respiratory transmitted ACE-2 receptor using Sarbeco Coronaviruses belong to a larger group of Beta-Coronaviruses naturally carried in bats, representing current and future threats. The global human COVID-19 pandemic is now witnessing the evolution of a great number of variants of SARS-CoV-2.

Vaccines that protect against SARS-CoV-2 and related Sarbeco-Coronaviruses have been developed using Digital Immune Optimised Synthetic Vaccine (DIOSynVax) technology developed in Cambridge. This broadly protective approach is needed for Coronaviruses such as SARS-CoV-2 and the SARS ACE2 receptor using Sarbeco Coronaviruses that have the potential to spill-over to humans from their animal reservoirs.

For the DIOS pan-Sarbeco vaccine candidate (pEVAC-PS), we have designed an antigen to induce immune responses against the Sarbeco Coronaviruses in order to protect against SARS-CoV-2, SARS-CoV and future outbreaks of related Coronaviruses. The majority of first wave SARS-CoV-2 vaccines in clinical trials and deployment are Spike (S) -based vaccines. There are two major concerns about single antigen S-based vaccine approaches due to the number of new circulating variants of concern (VOC); reduced vaccine efficacy, and the development of vaccine resistant strains.

The approach to design a pan-Sarbeco Coronavirus vaccine included structure-informed, computationally generated antigen libraries that were screened, selecting those conserved sub structures that elicit potent, specifically-targeted effector immune responses. High throughput screens, *in vivo* pre-clinical immune selection and vaccine efficacy readouts, which were down-selected to pEVAC-PS, an optimised-RBD vaccine capable of neutralising SARS-CoV-2 (2019), SARS-CoV-1 (2003), and other bat Sarbeco Coronaviruses.

DNA offers several potential advantages in that DNA vaccines are thermostable, are relatively straight-forward to manufacture and can be delivered using a needle-free device.

1.2. Study Rationale

Vaccines that protect against multiple related viruses have been developed using DIOSynVax technology. This broadly protective approach is needed for Coronaviruses such as SARS-CoV-2 and SARS-CoV, two of numerous related viruses that have the potential to cause future outbreaks from zoonotic reservoirs. The pEVAC-PS vaccine was designed to induce highly focussed immune responses against key neutralising antibody targets of the Sarbeco Coronaviruses in order to protect against SARS-CoV-2, SARS-CoV and potential future outbreaks of related Coronaviruses. pEVAC-PS is well-positioned to boost existing, licenced SARS-CoV-2 vaccines and to induce SARS-CoV antibody responses.

DNA sequences encoding the antigens are optimised for expression in mammalian cells before inserting into our DNA plasmid expression vector pEVAC. This vector is a flexible vaccine platform and any combination of antigens can be inserted to produce a different vaccine. DIOSynVax antigen inserts are engineered to be readily transferable to other scale-able vaccine delivery systems, such as Adenoviral or mRNA vectors.

This study will use an established needleless vaccine delivery technology to assess the optimal dose required to trigger patients' cells to produce the antigens, be recognised by participants immune systems and boost antibody titres that will induce durable protection against SARS-CoV-2 and SARS-CoV. Combined with needleless and powerless (no electrical source required) delivery, this platform offers a safe, widely deployable pan-Sarbeco Coronavirus vaccine for large scale immunisation programmes.

1.3 pEVAC-PS Vaccine

1.4 Pre-clinical studies of pEVAC-PS

1.5. Dose Rationale

The CE-marked PharmaJet Tropis intradermal (ID) delivery of DNA has demonstrated to be highly effective at reducing the dose required for anti-viral responses in several different systems. An adaptive dose escalation regime follows to discover the lowest dose of DNA

which will produce an effective immune response when delivered intradermally, with potential doses of 1.2mg, 0.8mg, 0.4mg and 0.2mg.

2. Objectives and Endpoints

Objective	Outcome measure
Primary To assess the safety and reactogenicity of a new pan-Sarbeco Coronavirus DNA candidate vaccine pEVAC-PS in a boosting regime in healthy volunteers.	a) Occurrence of solicited local reactogenicity signs and symptoms for 7 days following vaccination; b) Occurrence of solicited systemic reactogenicity signs and symptoms for 7 days following vaccination; c) Occurrence of unsolicited adverse events (AEs) for 28 days following vaccination; d) Change from baseline for safety laboratory measures and; e) Occurrence of disease enhancement episodes
Secondary To assess the humoral immunogenicity of pEVAC-PS when administered to healthy volunteers as a booster vaccine, through the analysis of SARS-CoV and SARS-CoV-2 RBD antibody titres. To assess the humoral immunogenicity of the candidate vaccine pEVAC-PS at 28 days (4 weeks) after 2 doses of pEVAC-PS have been administered 28 days apart, given by needle-free technology (PharmaJet); both administered in split doses, left and right deltoids (when >0.2mg)	a) Serology: receptor binding domain (RBD) responses in the majority of vaccinees per group for SARS-CoV and SARS-CoV-2
Secondary Assess optimum dosing of candidate vaccine pEVAC-PS	a) Compare dose tolerability and results of immunogenicity of up to four different doses of PharmaJet ID

	administration at 28 days (4 weeks) after the second dose of vaccine
Secondary **To assess long term (up to 12 months)** humoral immunogenicity of the candidate vaccine pEVAC-PS administered by the needle-free technology (PharmaJet) administered as 2 doses (split L&R when >0.2mg)	a) Serology: receptor binding domain (RBD) for SARS-CoV and SARS-CoV-2 RBD pre and post-immunisation.

3. Study Design

3.1. Study Design Overview

This is an adaptive, open label phase I dose escalation study of the safety, tolerability, immunogenicity and intra-dermal administration dose finding for the pEVAC-PS vaccine candidate against SARS-CoV and SARS-CoV-2. This study will recruit up to 36 healthy volunteers in up to 3 stages. All volunteers recruited will be healthy, male or non-pregnant female adults aged between 18 and 50 years who have been vaccinated no less than 12 weeks prior with 2 doses of a SARS-CoV-2 vaccine and have no history of infection and are seronegative for N.

The sentinel participant in each group will be observed for 1hr post vaccination and contacted 24hrs afterwards by the medical team to review symptoms and check against symptom diary. If no safety concerns are identified (section 7.9.1), the subsequent 5 participants can be vaccinated a minimum of 1 hour apart. Each participant will be observed for 60 minutes post vaccination. After review of participants in group 2a at 72hrs post dosing with the second immunisation, if no stopping criteria have been met (section 7.9) then the sentinel participant at the next dose level in 2b can be vaccinated.

Figure 1 (page 19) shows our approach to dose escalation to maximise participant numbers in groups that may inform future large scale clinical trials.

Stage 1

Group 1 will consist of 6 volunteers receiving 2 administrations of the lowest dose of ID injection at 0.2mg. There will be a review of the immunogenicity data 4 weeks following the second administration prior to proceeding to stage 2.

Stage 2

Group and dose allocation in stage 2 will depend on the results of the safety and immunogenicity analysis in stage 1, according to pre specified target immunogenicity outcomes.

a) Primary immunogenicity target met

Group 1 will be expanded with an additional 12 participants receiving 2 administrations of 0.2mg ID. If no safety issues are noted during expansion group and immunogenicity targets continue to be met, the study will be halted and a further review will be conducted by the study team to determine a reduced dose formulation and study regime, which will follow as a planned amendment to this protocol.

b) Primary immunogenicity target not met

2 additional groups consisting of 9 participants will be enrolled. Group 2a will receive 2 administrations of 0.4mg ID, and group 2b will receive 2 administrations of 0.8mg ID.

c) Very Poor/ No response

2 additional groups consisting of 9 participants will be enrolled. Group 2a will receive 2 administrations of 0.8mg ID, and group 2b will receive 2 administrations of 1.2mg ID.

There will be a review conducted of the safety and immunogenicity data 4 weeks after the second administration of vaccine for groups 2a and 2b to determine how to proceed in Stage 3.

Stage 3

If primary immunogenicity target is met during stage 2, then the group with the optimum immunogenicity and reactogenicity profile from Groups 2a or 2b will be expanded with an additional 12 participants in Group 3.

If the target is not met from group 2a or 2b (0.8mg), then an additional 12 participants will be enrolled into group 2c, who will receive 2 administrations of 1.2mg ID.

3.1.1. Immunogenicity endpoints

The adaptive protocol is based on reviews of immunogenicity data which will be made 4 weeks following the second administration of pEVAC-PS (8 weeks following the first dose). The decision for how to proceed will be made on the basis of the proportion of participants within each group who have seroconverted. Seroconversion will be defined as a positive readout from the SARS-CoV and/or SARS-CoV-2 RBD ELISA. The dose used for each group will be classified as either having met immunogenicity target, failed to meet immunogenicity target, or had very poor / no response. Proportions of participant seroconversion will be classed as the following:

Stage 1 – Meets immunogenicity target if 5 or 6 out of 6 participants have at least a 2-fold increase in Ab titre (at 0.2mg) we proceed to Expanded Group 1.

- **Stage 2** (Expanded Group 1) – if 12/12 participants have a 2-fold increase in Ab titre (at 0.2mg) consider to re-formulate and reduce the dose for a new study.
- **Stage 2** (Expanded Group 1) – if 10 or more of the 12 participants have at least a 2-fold increase in Ab titre (at 0.2mg) we select 0.2mg as the optimum dose to move forward into a Phase II study.
- **Stage 2** (Expanded Group 1) – if <=9 participants have a 2-fold increase in Ab titre (at 0.2mg) then a further 9 participants will be recruited to group 2a (0.4) and 2b (0.8mg) each, to be run in parallel.
    - The study will conclude after running groups 2a and 2b.

Stage 1 – Fails to meet immunogenicity target if only 3 or 4 out of 6 participants have at least a 2-fold increase in Ab titre, then we proceed to Groups 2a (0.4mg) and 2b (0.8mg) in parallel.

- **Stage 2** – if 7-9 or more participants have a 2-fold increase in Ab titre (at 0.4 or 0.8 mg) we expand whichever group has the higher number of responders. If both groups 2a and 2b have 9 responders, we will expand the lower dose.
- **Stage 2** – if <7 participants have a 2-fold increase in Ab titre (at 0.4 or 0.8 mg) we move to Stage 3 and increase dose to 1.2mg.

Stage 1 – With a poor response, if 2 or less participants out of 6 have a 2-fold increase in Ab titre, we proceed to Groups 2a (0.8mg) and 2b (1.2mg) in parallel.

- **Stage 2** – if 7-9 participants have a 2-fold increase in Ab titre (at 0.8 or 1.2mg) we expand whichever group has the higher number of responders.
- **Stage 2** – if <7 participants have a 2-fold increase in Ab titre at 0.8mg and 1.2mg the trial will conclude.

3.2. Study Population

Healthy volunteers aged 18-50 years (male or non-pregnant females) who are seronegative for previous SARS-CoV-2 infection (no anti-N SARS-CoV-2 responses or history of exposure). All groups will be recruited and vaccinated at the NIHR CRF, Southampton. Only subjects who meet all inclusion criteria and none of the exclusion criteria (specified in **Section 4**) will be allowed to participate in this study. The selection criteria include adequate provisions to minimize the risk and protect the well-being of subjects in the study.

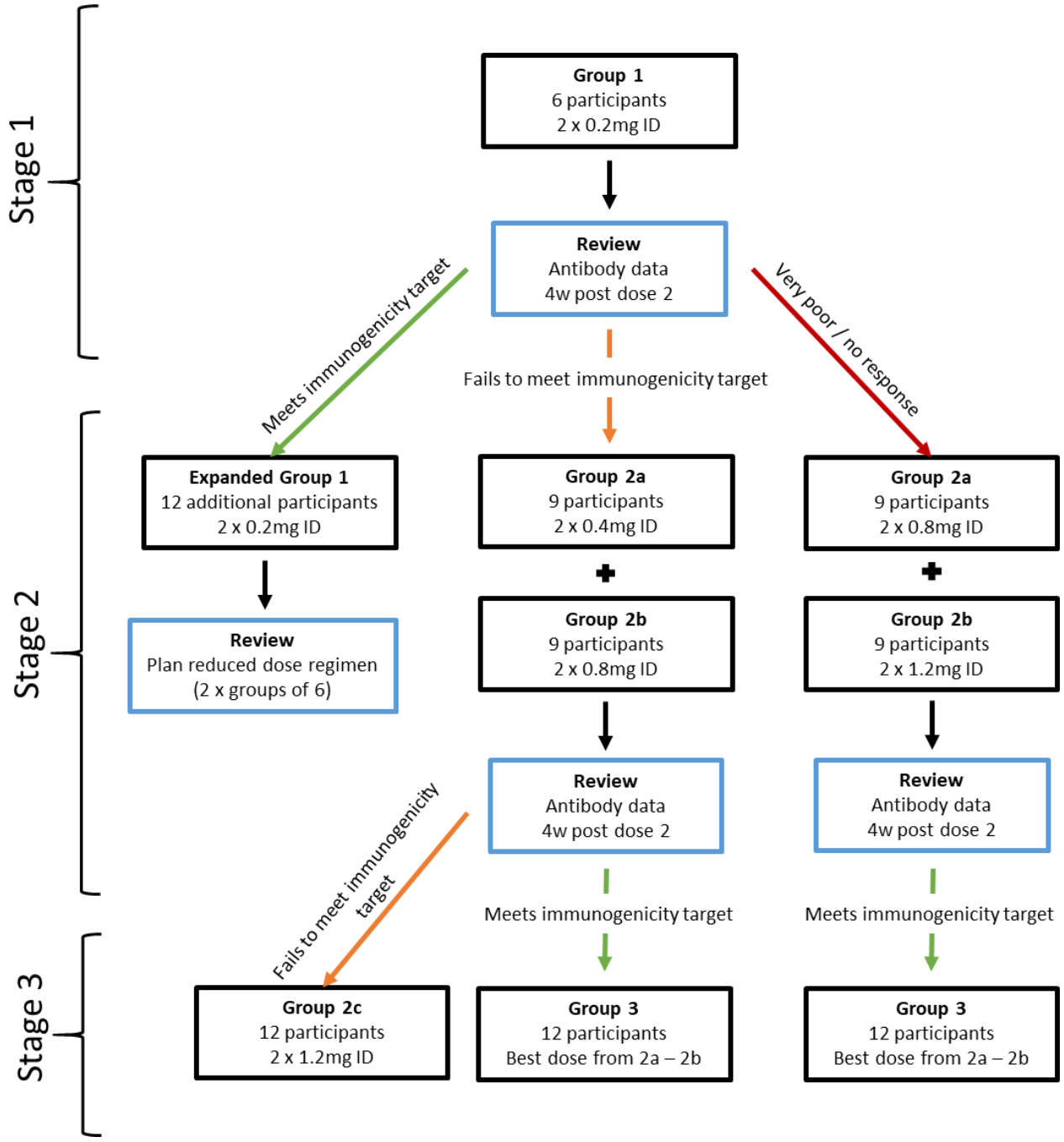
3.3. Description of Study Cohorts

There will be up to study groups in the trial, with a total of up to 36 volunteers.

Table 1: Study groups.

Stage 1	**Group 1a**	6 volunteers; 2 doses of pEVAC-PS at 0.2mg intradermally on Day 0 and Day 28.
Stage 2	**Subgroup 1a-expanded**	12 volunteers; 2 doses of pEVAC-PS at 0.2mg intradermally on Day 0 and Day 28.
	Group 2a	9 volunteers; 2 doses of pEVAC-PS at 0.4mg OR 0.8mg intradermally on Day 0 and Day 28.
	Group 2b	9 volunteers; 2 doses of pEVAC-PS at 0.8mg OR 1.2mg intradermally on Day 0 and Day 28.
Stage 3	**Group 2c** If the target is not met from group 2a or 2b (0.8mg)	12 volunteers; 2 doses of pEVAC-PS 1.2mg intradermally on Day 0 and Day 28
	Group 3 If primary immunogenicity target is met during Stage 2	12 volunteers; 2 doses of pEVAC-PS at the most well tolerated and immunogenic dose from Group 2a or 2b on Day 0 and Day 28.
	If the immunogenicity targets are not met by 1.2mg in group 2b, no further participants will be enrolled.	

Figure 1. Study Flow Diagram



3.4 Number of Volunteers/Centres

The study will be conducted at one UK site, Southampton NIHR Clinical Research Facility. The target is to enrol up to 36 healthy volunteers (female and/or male).

3.5. Dose escalation

The sentinel participant in each group will be observed for 1 hr post vaccination for any local reactions and contacted 24hrs afterwards by the medical team to review symptoms and check against symptom diary. If no safety concerns are identified (section 7.9.1), the subsequent 5 participants can be vaccinated a minimum of 1 hour apart. Each participant will be observed for 60 minutes post vaccination. After review of participants at 72hrs post dosing, if no stopping criteria have been met (section 7.9) then the procedure can be repeated with the first subject at the next dose level.

3.5.1. Rationale and Design of Dose optimisation Strategy

Current formulation constraints mean that the minimum dose of pEVAC-PS which can be administered intradermally is 0.2mg, and the maximum is 1.2mg. An adaptive protocol allows optimisation within this dosing range with potential to expand groups which achieve desirable immunogenicity and reactogenicity profiles.

Sufficient immune responses are expected from significantly lower doses administered intradermally given the increased numbers of antigen presenting cells present within the dermis as compared to muscle cells.

The adaptive design from intradermal administration starts at the lowest currently achievable dose. If that is successful, this dosing group will be expanded, and the team will review the potential for additional groups receiving lower doses (this will require adaptation of the formulation).

If primary immunogenicity endpoints are not met, successive groups will be enrolled at higher doses until the pre specified immunogenicity target is met. At this point, the group with the optimum immunogenicity and reactogenicity profile will be further expanded.

4. Participant Eligibility Criteria

4.1. Inclusion Criteria

The healthy adult volunteers must meet all the following inclusion criteria to be eligible for the study:

- Healthy adults aged 18-50 years.
- Able and willing (in the Investigator's opinion) to comply with all study requirements (participants must not rely on public transport or taxis).
- Willing to allow the investigators to discuss the volunteer's medical history with their General Practitioner and access all medical records when relevant to study procedures.

- Agreement to refrain from blood donation during the course of the study.
- Provide written informed consent.
- Completed a 2 dose primary immunisation course against COVID-19, with the most recent dose administered no less than 12 weeks (84 days) previously

Female volunteers of childbearing potential are required to use an effective form of contraception at enrolment and until after 3 months following their last personal dose of vaccination within the study. A woman of childbearing potential is defined as a pre-menopausal female who is capable of becoming pregnant. Menopause can be diagnosed in a woman aged over 50 after one year of amenorrhoea (this applies only if the woman is not using hormonal contraception).
Acceptable forms of contraception for female volunteers are as follows:

- Established use of oral, injected or implanted hormonal methods of contraception
- Placement of an intrauterine device or intrauterine system
- Total abdominal hysterectomy or surgical sterilisation
- 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

4.2 Exclusion criteria

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

- History of laboratory confirmed MERS, SARS-CoV-1 or SARS-CoV-2 infection
- Seropositive for SARS-CoV-2 Nucleocapsid IgG at screening
- Have an anti-SARS-CoV-2 and a SARS-CoV-1 RBD response of more than 2 AUC (Area Under the Curve) units
- Planned receipt of any vaccine other than the study intervention within 30 days before and after each study vaccination.
- Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration of the vaccine candidate.
- Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent severe infections and use of immunosuppressant medication within the past 6 months, except topical steroids or short-term oral steroids (course lasting <14 days).
- Any autoimmune conditions, except mild psoriasis, well-controlled autoimmune thyroid disease, vitiligo or stable coeliac disease not requiring immunosuppressive or immunomodulatory therapy.
- History of allergic disease or reactions likely to be exacerbated by any component of the pEVAC-PS vaccine
- Any history of angioedema.
- Any history of anaphylaxis.
- Pregnancy, lactation or willingness/intention to become pregnant for 3 months following the last personal dose of vaccine in this trial.

- History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ).
- History of serious psychiatric condition likely to affect participation in the study (e.g. ongoing severe depression, history of admission to an in-patient psychiatric facility, recent suicidal ideation, history of suicide attempt, bipolar disorder, personality disorder, alcohol and drug dependency, severe eating disorder, psychosis, use of mood stabilisers or antipsychotic medication).
- Bleeding disorder (e.g. factor deficiency, coagulopathy or platelet disorder), or prior history of significant bleeding or bruising following IM injections or venepuncture.
- Any other serious chronic illness requiring hospital specialist supervision.
- Chronic respiratory diseases, including mild asthma (resolved childhood asthma is allowed)
- Chronic cardiovascular disease (including hypertension), gastrointestinal disease, liver disease (except Gilberts Syndrome), renal disease, endocrine disorder (including diabetes) and neurological illness (excluding migraine)
- Seriously overweight (BMI≥40 Kg/m^2) or underweight (BMI≤18 Kg/m^2)
- Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 42 units every week.
- Suspected or known injecting drug abuse in the 5 years preceding enrolment.
- Any clinically significant abnormal finding on screening biochemistry, haematology blood tests or urinalysis.
- 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.

5. Conduct of the study

5.1 Volunteer Identification

Healthy volunteers aged 18-50 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 the screening process.

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 institutional websites where information will be given and the volunteer information sheet will be downloadable or sent to the volunteer upon request
- 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
- As a post on a Twitter, Facebook or other social media account owned and operated by the study research groups

- 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 Southampton NIHR-CRF
- 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 2018. Investigators would not be given dates of birth or ages of individuals but the list supplied would only contain names of those aged between 18-50 years (as per the inclusion criteria).
- Southampton NIHR-CRF Database of Healthy Volunteers: We may contact individuals from this database who have previously expressed an interest in receiving information about future studies for which they may be eligible

Volunteers will be sent a copy of the participant information sheet in response to requests for further information. Volunteers will be given a minimum of 24 hours to review the documentation prior to attending a screening visit. A pre-screening call will then be undertaken for those volunteers that still show interest in the study after receiving the PIS. This is one of the standard procedures used by the site for determining basic eligibility for healthy volunteer studies in order to ensure potential participants do not have their time wasted coming for a face to face screening visit if they are definitely not eligible to participate.

5.2 Schedule of events

Table 2. Schedule of events

Attendance Number	1[S]	2	3	4	5	6	7	8	9	10	11	12[†]	COVID-19 Testing
Timeline** (days)	≤ 90	0	1	3	7	14	28	42	56	84	182	365	As required
Time window (days)				±1	±2	±3	±7	±3	±7	±3	±14	± 28	N/A
Informed Consent	X												
Review eligibility criteria	X	X					X						
Vaccination (IM)		X					X						
Vital signs^	X	X		X	X	X	X	X	X	X	X	X	X
Height and weight	X												
Telephone/Video call			X										
Ascertainment of adverse events and con meds		X	X	X	X	X	X	X	X	X	X	X	X
Diary cards provided		X					X						
Diary cards reviewed			X	X	X	X	X	X	X				
Medical History, Physical Examination	X	(X)		(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
Biochemistry, Haematology (mL)	5	5		5	5		5	5					5
Exploratory immunology (mL)		50			50	50	50	50	50	50	50	50	up to 50
Nose/Throat Swab													X
Urinalysis	X												
Urinary bHCG (women only)	X	X					x						
HBsAg, HCV Ab, HIV serology (mL)	5												
Blood volume per visit	10	61.5		5	55	50	55	55	50	55	50	50	up to 57.5
Cumulative blood volume[%]	10	71.5		76.5	131.5	181.5	236.5	291.5	341.5	396.5	446.5	496.5	554

S=screening visit; (X) = if considered necessary ^ = Vital signs includes pulse, blood pressure and temperature; ** Timeline is approximate only. Exact timings of visits relate to the day on enrolment, i.e., each visit must occur at indicated number of days after enrolment ± time window. % Cumulative blood volume for volunteers if blood taken as per schedule, and excluding any repeat safety blood test that may be necessary. [†]Only for the final group which are enrolled

Table 3: Schedule of Events for Unscheduled and Early Termination Visits. Applicable for both all groups

	Unscheduled Visit Following AE	**Early Termination Visit**
Medical History	(x)	(x)
Physical Examination	(x) [1]	(x)
ECG	(x)	
Urinalysis	(x)	
Physical Observations	x	x
Concomitant medications	x	x
AEs reviewed	x	x
Diary card review[2]	x	(x)
Haematology (mL)	3	3
Biochemistry (mL)	5	5
Coagulation (mL)	(2.7)	(2.7)

(x) = If considered necessary, emphasising any acute complaints.

[1] A full physical examination or symptom directed physical examination can be carried out at the discretion of the investigator.
[2] Required if the participant is currently within the diary card completion phase of the study only.

The above schedule is not an exhaustive list; procedures carried out will be guided by clinical need and will be at the discretion of the investigator.

5.3 Study Procedures

5.3.1. Medical History

A detailed medical history will be taken at set study visits as outlined in the schedule of events, including recent acute illness or events which could constitute AEs.

5.3.2. Physical examination

A detailed physical examination including review of vital systems will be performed at set study visits as outlined in the schedule of attendances. On occasions where a physical exam is carried out as determined by clinical need, these will be either a full physical examination or symptom directed physical examination at the discretion of the investigator.

5.3.3. Physical Observations

Pulse, blood pressure and body temperature will be measured at the time points indicated in the schedule of attendances. Height and weight will be measured at screening only.

5.3.4 Concomitant Medications

Concomitant medications administered up to 30 days before screening must be recorded in the eCRF at the screening visit. Concomitant medications will be recorded prior to vaccine administration at vaccination visits and at each follow up visit. Volunteers will be asked to record any medication that they have taken in their diary card for 28 days post each vaccine administration.

Receipt of any IMP (including experimental vaccines other than the study vaccine) during the study is not allowed.

5.3.5. Blood Tests

Blood will be drawn for the following laboratory tests and processed:

1. At University Hospital Southampton NHS Foundation Trust Laboratories, using NHS standard procedures:

- **Haematology;** Full Blood Count.
- **Biochemistry;** Sodium, Potassium, Urea, Creatinine, Albumin, Liver Function, Magnesium and Cholesterol.
- **Diagnostic serology;** HBsAg, HCV antibodies, HIV antibodies (specific consent will be gained prior to testing blood for these blood-borne viruses).
- **β-HCG serum** (female volunteers only)

2. At LVZ laboratories:

Immunology: Serological analysis of antibody responses (such as ELISA); B cell assays. Other immunological assays including flow cytometry, pseudotype neutralisation for Sarbeco viruses, amongst others, and T-cell assays may be performed at the discretion of the Investigators.

5.3.6. Urinalysis

Urine will be tested for blood, protein and glucose at screening. For female volunteers only, urine will be tested for beta-human chorionic gonadotrophin (HCG) at screening and immediately prior to each vaccination.

5.3.7. Pregnancy testing

For female volunteers only, a serum beta-human chorionic gonadotrophin (β HCG) test will be carried out at screening and urine will be tested for β HCG prior to each vaccination.

5.3.8. Administration of vaccines

Before each vaccination, the on-going eligibility of the volunteer and contraindications will be reviewed. The vaccine will be administered as described in **Section 6.3** *'Dosage and administration of study vaccine'.* The injection site will be covered with a sterile dressing and the volunteer will stay in the clinical area for 60 minutes (+15/-5 minutes) post vaccination. The sterile dressing will be removed and injection site inspected at 30 minutes (+15/- 5 minutes)

post vaccination. Observations will be taken at 30 minutes (+/- 5 minutes) post vaccination and at 60 minutes (+15/-5 minutes) post vaccination.

5.3.9. Distribution, review and completion of diary cards

Volunteers will be asked to complete a diary card for a period of 2 months following administration of vaccine on Day 0. A separate diary will be issued for each 28 day period post vaccination. An oral thermometer, tape measure and access to a paper diary card for solicited and unsolicited AEs will be given to each volunteer. The diaries will collect information on the timing and severity of the following solicited AEs:

Table 4: Solicited AEs as collected on post vaccination diary cards

Local solicited AEs	**Systemic solicited AEs**
Pain	Fever
Redness	Feverishness
Warmth	Joint pains
Itch	Muscle pains
	Fatigue
	Headache
	Nausea
	Malaise

Volunteers will be instructed on how to self-assess the severity of these AEs. There will also be space on the diary card to self-document unsolicited AEs, and whether medication was taken to relieve the symptoms.

Volunteers will be asked to bring their diary to the study site at each follow up visit. Diaries will be reviewed by a member of the study team. All diaries will be collected at the Week 8 follow up visit.

5.3.10. Distribution of Emergency contact card

Volunteers will be provided with an emergency 24-hour telephone number to contact the on call study physician if needed. In an emergency situation this card will be used to inform the attending physician that the subject is in a clinical study and that relevant information can be obtained by contacting the study investigator. Volunteers will be instructed to keep this card on their person at all times for the duration of their participation in the study.

5.3.11. Clinical Reviews

All clinical reviews and procedures will be undertaken by a member of the study team. The procedures to be included in each visit are documented in Tables 2 and 3. Each review is assigned a time point and a window period within which the review will be conducted.

5.4 Screening Visit

5.4.1. Informed Consent

It is the responsibility of the Principal Investigator or co-investigator (where the responsibility has been delegated by the Principal Investigator as captured on the Site Signature and Delegation Log) to obtain written informed consent for each volunteer before performing any trial related procedure.

The study information sheet will be made available to the volunteer at least 24 hours prior to the screening visit.

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.
- 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 SARS-CoV-2, 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 risks of participating in the study will be fully explained
- Study samples and data might be shared with other institutions within the UK and Europe, investigating the immune response to SARS-CoV-2. These will be anonymised.
- Study samples and data will be stored indefinitely either as part of an ongoing ethically approved study or in a human tissue authority (HTA) licensed biobank.
- Volunteers will be given the option to be contacted in the future for possible follow up trials looking at COVID-19

The aims of the study and all tests to be carried out will be explained. The volunteer will be given the opportunity to ask any questions that they have and will then have time to consider whether or not to participate. If they do decide to participate, they will sign and date the consent form and will be given a copy to keep. The original consent form will be stored as source documentation. These forms will also be signed and dated by the Investigator.

Details of the informed consent discussion will be recorded in the volunteer's medical record. This will include the date consent was given, with the name of the trial and the version number of the Participant Information Sheet and Informed Consent Form. Throughout the trial the volunteer should have the opportunity to ask questions about the trial and any new information that may be relevant to the volunteer's continued participation should be shared with them in a timely manner. On occasion it may be necessary to re-consent the volunteer, for example if new information becomes available or an amendment is made to the protocol that might affect the volunteer's participation in the trial. In this case the process above will be followed and the volunteer's right to withdraw from the trial respected.

5.4.2. Screening Clinical Assessments

No trial specific procedure should be carried out prior to signing the consent form for this study. All potential volunteers will have a screening visit, which may take place up to 90 days prior to vaccination. Informed consent will be taken before screening, as described in **section 5.4.1.** If consent is obtained, the procedures indicated in the schedule of events will be undertaken including a medical history, physical examination, blood tests, and urine test. To avoid unnecessary additional venepuncture, if the appropriate blood test results for screening are available for the same volunteer from a screening visit for another study, these results may be used for assessing eligibility (provided the results date is within the 3 months preceding administration of the first dose of pEVAC-PS).

Abnormal clinical findings from the medical history, physical examination and all tests at screening will be assessed by a clinician referring to site-specific laboratory adverse event grading tables which are filed in the trial master file (TMF). Any abnormal test result deemed clinically significant may be repeated to ensure it is not a single occurrence. If an abnormal finding is deemed to be clinically significant, the volunteer will be informed and appropriate medical care arranged with the permission of the volunteer.

The eligibility of the volunteer will be reviewed by the study clinician at the end of the screening visit and again when all results from the screening visit have been considered and a copy of the volunteer's medical history has been received from the GP. Decisions to exclude the volunteer from enrolling in the trial or to withdraw a volunteer from the trial will be at the discretion of the Investigator. If eligible, a day 0 visit will be scheduled for the volunteer to receive the vaccine.

5.4.3. Study Identifier Code (SIC) Allocation

Volunteers will be allocated a 3-digit subject identifier code (SIC). This will be allocated by the site reflecting the order of consent to the study within the site. For example, the third subject who signed an informed consent form at the study site will be identified as Subject 003. All study documents (e.g. CRFs, clinical documentation, sample containers, drug accountability logs, etc.) will be identified with the SIC.

The site study team will add the patient's allocated SIC to the site screening & recruitment log and keep it in the Investigator Site File.

5.4.4. Request of Medical History from GP

The volunteer's GP will be contacted to corroborate their medical history. Volunteers will only be enrolled in the study if written or verbal information regarding the volunteer's medical history is obtained from the GP. In cases where verbal consent is given, the study investigator conducting the telephone call will document the discussion and this will be filed in the volunteer's medical notes.

5.4.5. The Over-Volunteering Prevention System (TOPS)

Volunteers will be excluded from the study if they are concurrently involved in another trial. In order to check this, volunteers will be asked to provide their National Insurance or Passport number (if they are not entitled to a NI number) and will be registered on a national database of participants in clinical trials (*www.tops.org.uk*).

5.5 Participant visits

5.5.1 Vaccination Visits

Following review of safety blood results and GP medical history, eligible subjects will be booked to attend a vaccination visit on Day 0. After re-checking inclusion and exclusion criteria, an abbreviated physical examination (at the investigator's discretion) and measurement of physical observations, subjects will be enrolled into a study group for vaccination. Volunteers will be enrolled into each group or subgroup sequentially. Volunteers will be informed of which group they will be enrolled into. Volunteers will not be randomised into a group in this study.

If medical status and/or physical examination suggest significant changes have occurred since screening, the Day 0 visit can be re-scheduled, or the subject excluded from the study if he/she fails to meet the inclusion and exclusion criteria. The study team will advise the volunteer prior to the dosing visit that receipt of licensed vaccines e.g. (FLU vaccine) is not permitted within the month preceding vaccination or the month following each vaccination. Should a volunteer have received a licensed vaccine in the month prior to pEVAC-PS vaccination the Day 0 visit will be rescheduled to an acceptable time-point.

Before vaccination, the investigator must check for any symptoms of an acute illness or body temperature ≥37.5°C. In such a situation, the subject may be vaccinated at a later date within the screening window or be withdrawn at the discretion of the investigator. The vaccine will be administered as detailed in **Section 6.3** *'Dosage and administration of study vaccine'.*

Following vaccine administration, subjects will be observed for a minimum of 60 minutes for the development of any acute reactions, or longer if deemed necessary.

Subjects will be provided with a subject diary, thermometer, and ruler to measure and record body temperature, solicited local and systemic AEs.

This will be repeated on Day 28 for administration of the second dose of vaccine.

If the volunteer has remained well during this observation period, they may go home and will receive a follow up telephone call the next day.

Any significant safety concerns that have arisen during the observation period will be communicated to the CI and local safety monitor. An extended period of observation will be instigated if necessary. In this unlikely event, the following procedures will be followed as a minimum:

- The volunteer will be accompanied in the observation area by a trial nurse or study investigator. The frequency of physical observations (blood pressure, pulse, temperature) will be determined by the CI, for example hourly (+/- 15 minutes).
- The trial clinician may initiate basic treatments for vaccine related symptoms including anti-pyretic and anti-emetic medication.
- Intravenous fluid can also be given for tachycardia or hypotension of grade 2 or above or for any other reason of clinical significance according to the trial investigators judgement.

If at any point following vaccination, the CI or local safety monitor identifies any acute clinical concern requiring urgent medical intervention, an acute admission for further clinical management will be arranged.

5.5.2 Subsequent Visits

Volunteers will return to the study site for follow up visits as specified in Table 2 and Table 3. Diary cards will be reviewed for details of solicited and unsolicited AEs for 28 days after each vaccination, and any new or undocumented medical issues or symptoms that have arisen will be assessed. Physical observations and venepuncture for immunology and safety bloods will be undertaken as per the applicable schedule of events. Information on medications administered will be recorded. On Day 1 volunteers that have been enrolled in Group 1 will be contacted via telephone to collect safety information (solicited and unsolicited AEs, SAEs and concomitant medications).

5.5.3 Participants under quarantine

Given the evolving epidemiological situation both globally and in the UK, should a participant be under quarantine and unable to attend any of the scheduled visits, a telephone/video consultation will be arranged using smartphone or computer app if clinically appropriate in order to obtain core study data where possible.

Participants with confirmed SARS-CoV-2 infection (COVID-19 Pathway)

Participants will be counselled at enrolment that should they receive a positive SARS-CoV-2 test (e.g. an antigen detection or nucleic acid amplification test, for example, via test and trace or occupational health services) they should contact the trial team on receipt of the positive result. A study doctor will be available to direct the volunteer for routine medical care via standard NHS pathways should that be appropriate, depending on the volunteer's clinical condition.

This COVID-19 pathway will apply to participants tested via symptomatic and asymptomatic pathways.

Once the participant has conveyed their result to the study team, and the study team confirm an appropriate test has been used (verbal discussion with participant as to how testing was obtained, confirmatory documentation will not be sought), an appointment will be arranged to review the participant. At this visit blood samples for safety (FBC, Biochemistry, CRP and others if deemed clinically relevant) and immunology (PBMCs and serum for cellular and humoral immune responses) will be taken, along with a nasopharyngeal swab for storage and subsequent viral isolation. Vital signs and other clinical data will be recorded.

Participants will be eligible if they can attend the site for a COVID-19 visit within 7 days of a positive test.

5.6 Discontinuation/withdrawal of study volunteers

The following medical occurrences associated with vaccine immunisation constitute absolute contraindications to further administration of vaccine. If any of these events occur during the study, the subject must be withdrawn and followed until resolution of the event, as with any adverse event:

- Anaphylactic reaction following administration of vaccine
- Pregnancy

The following adverse events constitute contraindications to administration of vaccine at that point in time; if any one of these adverse events occurs at the time scheduled for vaccination, the subject may be vaccinated at a later date, or withdrawn at the discretion of the Investigator. The subject must be followed until resolution of the event as with any adverse event:

- Acute disease at the time of vaccination. (Acute disease is defined as the presence of a moderate or severe illness with or without fever). All vaccines can be administered to persons with a minor illness such as diarrhoea, mild upper respiratory infection with or without low-grade febrile illness, i.e. temperature of ≤37.5°C/99.5°F.
- Temperature of ≥37.5°C (99.5°F) at the time of vaccination.

In accordance with the principles of the current revision of the Declaration of Helsinki 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. The Investigator may withdraw the volunteer at any time in the interests of the volunteer's health and well-being. In addition, the volunteer may withdraw/be withdrawn for any of the following reasons:

- Administrative decision by the Investigator.
- Significant protocol deviation.
- Volunteer non-compliance with study requirements.
- An AE, which requires discontinuation of the study involvement or results in inability to continue to comply with study procedures.
- Pregnancy

The reason for withdrawal will be recorded in the eCRF. If withdrawal is due to an AE, 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 is withdrawn from the study may be replaced, if that is possible within the specified time frame. The Local Safety Monitor (LSM) may recommend withdrawal of volunteers. Any volunteer who fails to attend for two or more follow-up visits during the study, despite active attempts to contact volunteer by trial team, will be deemed to have withdrawn from the study. The study team will make reasonable attempts to contact volunteers that do not return for scheduled visits or follow-up.

If a volunteer withdraws from the study, blood samples collected before their withdrawal from the trial will be used/ stored unless the volunteer specifically requests otherwise.
In all cases of subject withdrawal, except for those of complete consent withdrawal, long-term safety data collection including some procedures such as safety bloods, may continue as appropriate if subjects have received one or more vaccine doses and have indicated willingness to attend a relevant follow-up time point. Should a volunteer decide that they do not want to proceed with receiving a second dose of pEVAC-PS after receiving the first dose but agree to continue attending follow up visits they will be asked to attend at Day 1 (telephone call), Day 3, Day 7, Day 14, Day 28, Month 3 and Month 6.

5.6.1 Volunteer Replacement

The investigator and local safety committee will assess whether any volunteers that withdraw from the study after enrolment will need to be replaced. All existing safety and adverse event data from withdrawn participants will be provided to the local safety committee as part of the safety report.

5.7 Duration of Study

Volunteers will be considered to be enrolled in the study on receipt of their first vaccination. Volunteers will remain in the study for a maximum of 18 months (+/- 28 days) after being enrolled (this includes up to 6 months during the recruitment and dosing phase with a follow-up period of up to 12 months).
The study is considered to be complete upon last volunteer/last visit at the site. The end of study is defined as the completion of the testing of samples, to be achieved no later than 12 months after the date of the last visit of the last volunteer.

5.8 Sample handling

5.8.1. Laboratory work

This immunogenicity study will be conducted in laboratories at the Lab of Viral Zoonotics, at the University of Cambridge. The laboratories that will be used are 207 and the equipment bay, with the North laboratory as a back-up facility. The ELISA will be carried out in 207.

5.8.2. Labelling of samples

As the clinical trial is open label, the serology lab (LVZ) at the University of Cambridge will be able to identify which samples are from which treatment group. Each blood sample will be labelled with subject number (e.g. 001), study protocol number (RHM MED 1776), study day (e.g. S, Day 0, 7, 14) and the date the sample was taken. The secondary container in which samples are shipped to LVZ will state the required storage conditions and will specify the nature of the samples (i.e. heparinised blood / SST blood). If any additional information which may breach patient confidentiality is present on sample tubes, LVZ staff will operate in accordance with the relevant SOP in dealing with such samples.

5.8.3. Storage of samples

Blood samples will be processed into serum and PBMCs on the day of receipt at LVZ in accordance with local SOP or separated at the study site and shipped frozen to LVZ in batch. Processing of blood into serum and PBMCs will be carried out in line with local risk assessment.

Serum and PBMC samples will be aliquoted into cryotubes labelled by LVZ after preparation from blood. Labels will contain identical information to the blood tubes, but will also specify 'human serum' or 'human PBMCs' to denote the type of sample contained within the vial. Serum and PBMC vials will be numbered sequentially for each patient (e.g. S01, P01, P02) in accordance with local SOP. LVZ will only identify samples from these labels.

Once serum and PBMCs have been prepared, samples will be stored in cryoboxes below -60°C in accordance with SOP MIG/001. PBMCs may be transferred to liquid nitrogen for long-term storage. Serum samples will be removed from the ultra-low temperature (ULT) freezer as and when they are tested and will be returned to the ULT freezer after testing.

The samples will be kept until the clinical study report has been finalised. The sponsor will inform LVZ when the clinical study report has been finalised and whether samples are to be returned to the study site or are to be destroyed at LVZ. If the sponsor does not inform the LVZ what to do with the samples then LVZ will return any remaining aliquots to the study site after the clinical study report has been finalised.

5.8.4. Shipment of samples

Samples will be shipped to LVZ by courier at ambient temperature on the day of blood being drawn, within normal working hours or separated and frozen at the study site to be shipped in batch at a later date. A schedule of sampling dates will be prepared by the study site and agreed with LVZ in advance to ensure availability of staff for sample receipt and processing. Any changes to this sampling schedule will be communicated to and agreed with LVZ in advance.

Upon receipt, the blood samples will be taken to test facility laboratories where details on the sample tubes will be checked to ensure that samples received are as expected. Any discrepancies will be recorded on the blood sample shipment form. The original forms will be filed in the RHM MED1776 study test sample file. For each shipment, PDF copies of the completed forms will be e-mailed to the Chief Investigator at the study site to document receipt. This e-mail will also state the sample numbers and volumes received, the date of receipt and the nature of any discrepancies. This e-mail will also request documented approval to proceed

with testing of this batch of blood samples, once processed into serum/PBMCs. A batch of samples will only be tested at LVZ once written approval is received from the sponsor for that batch.

6. Investigational Product

6.1. Description (also see Investigator's Brochure [IB])

6.2. Storage and handling

The vaccine will be stored at -20°C (nominal temperature).
Maintenance of the correct storage conditions will be the responsibility of the site using an appropriately qualified and monitored storage unit. Any temperature excursions or deviations from the procedures outlined below will be reportable to the Chief Investigator and Sponsor QP. Vaccine accountability, storage, shipment and handling will be in accordance with supplier and study site SOPs. All movements of the study vaccines will be documented. All study vaccines to be administered to the subjects must be stored in a temperature monitored locked freezer with no access by unauthorised personnel.

6.3. Dosage and administration of study vaccine

The dose administered will be dependent on group and cohort allocation. Please see **Section 3.3 Table 1** for more information on dosing groups and the corresponding dose allocation. Instructions for dose preparation can be found in the IMP procedures manual.

Table 5: Dose and corresponding administrations per vaccination visit (total of two visits).

Delivery method	Total DNA (mg)	Left deltoid	Right deltoid
ID (Tropis)	0.2	0.1ml x1	-
ID (Tropis)	0.4	0.1ml x1	0.1ml x1
ID (Tropis)	0.8	0.1ml x2	0.1ml x2
ID (Tropis)	1.2	0.1ml x3	0.1ml x3

6.3.1 Intradermal injections

The vaccine will be administered intradermally using the PharmaJet Tropis device in the deltoid muscle of both arms (when dose is >0.2mg). During administration of the vaccines, Advanced Life Support drugs and resuscitation equipment will be immediately available for the management of anaphylaxis. At each vaccination visit, vaccines will be allowed to thaw to room temperature and should be administered as soon as possible. Although the components of the drug product formulation are compatible and stable for at least 24 hours after thawing, the investigator is encouraged to administer the formulation as soon as possible, and not beyond 2 hrs after removal from the freezer. If not used within 2hrs, the final formulated vial must be discarded. Depending on dose and concentration, one or more vials of vaccine may be used.

The injection site will be covered with a sterile dressing and the volunteer will stay in the Southampton NIHR CRF for observation, in case of immediate adverse events. Observations will be taken 30 minutes after vaccination (+/-5 minutes) and the sterile dressing removed and injection site inspected. Observations will also be taken at 60 minutes (+15/-5 minutes) before the volunteer leaves.

Study vaccine will be administered by a study vaccine administrator – a trained and qualified study nurse, medical doctor, or otherwise qualified health care professional. The date and time of each study vaccine administration will be recorded in the eCRF.

6.4. Vaccine supply

6.5. Vaccine accountability

A drug accountability log will be kept by the investigational pharmacist or designee, in which he/she will record the date(s) and quantity of all IMP dispensed for each volunteer. The inventory will be made available to the study monitor who will verify accountability and dose during the course and/or at the end of the study. All used and unused containers will be accounted for during the study and will either be returned to the sponsor for destruction or destroyed on site if approved by the sponsor (in this case a written confirmation of destruction must be provided).

7. Safety

7.1. Safety definitions

Adverse Event (AE)

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

Adverse Reaction (AR)

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

Unexpected Adverse Reaction (UAR)

An adverse reaction, the nature or severity of which is not consistent with the applicable information about the vaccine in the protocol, is considered as an unexpected adverse reaction.

Serious Adverse Event (SAE)

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

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

Serious Adverse Reaction (SAR)

An adverse event that is both serious and, in the opinion of the reporting investigator or sponsor, believed to be possibly, probably or definitely due to the inoculum or any other study treatments, based on the information provided in the protocol.

Suspected Unexpected Serious Adverse Reactions (SUSARs)

A SUSAR is a SAE that thought to be possibly, probably or definitely related to the inoculum and is unexpected in nature (when assessed against the IB).

7.2. Foreseeable Medical Occurrences

The foreseeable ARs following vaccination with pEVAC-PS include injection site pain, erythema, warmth, swelling and pruritus, myalgia, arthralgia, headache, fatigue, fever, feverishness, malaise and nausea. These adverse events will be listed as solicited adverse

events providing they occur within 7 days following the day of vaccination. AEs other than these, or those AEs occurring outside of the 7 days after vaccination, will be listed as unsolicited adverse events.

7.3 Expected Serious Adverse Events

There are no expected serious adverse events for this study.

7.4. Adverse Events of Special Interest

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

- Severe hypersensitivity reactions (e.g. Anaphylaxis)
- Any new, suspected auto-immune disease
- Myocarditis or pericarditis
- Blood clotting events associated with low platelets (thrombocytopaenic thrombosis)
- COVID-19 disease or SARS-CoV-2 infection

7.5. Evaluation of AEs, SAEs and other safety events

When an AE/SAE occurs, the investigator is responsible for reviewing all documentation related to the event. The investigator will record all relevant information on the eCRF and make an assessment on causality and severity.

7.5.1. Assessment of Causality

For every unsolicited AE, an assessment of the relationship of the event to the administration of the vaccine will be undertaken by the CI or the CI-delegated clinician at the site. An intervention-related AE refers to an AE for which there is a probable or definite relationship to administration of a vaccine. An interpretation of the causal relationship of the intervention to the AE in question will be made, based on the type of event; the relationship of the event to the time of vaccine administration; and the known biology of the vaccine therapy (Table 6). Causality assessment will take place during planned safety reviews, interim analyses (e.g. if a holding or stopping rule is activated) and at the final safety analysis, except for SAEs, for which causality should be assigned by the reporting Investigator.

Table 6: Guidelines for assessing the relationship of vaccine administration to an AE.

0	No Relationship	No temporal relationship to study product ***and*** Alternate aetiology (clinical state, environmental or other interventions); ***and*** Does not follow known pattern of response to study product
1	Unlikely	Unlikely temporal relationship to study product ***and*** Alternate aetiology likely (clinical state, environmental or other interventions) ***and*** Does not follow known typical or plausible pattern of response to study product.
2	Possible	Reasonable temporal relationship to study product; ***or*** Event not readily produced by clinical state, environmental or other interventions; ***or*** Similar pattern of response to that seen with other vaccines
3	Probable	Reasonable temporal relationship to study product; ***and***

		Event not readily produced by clinical state, environment, or other interventions ***or*** Known pattern of response seen with other vaccines
4	Definite	Reasonable temporal relationship to study product; ***and*** Event not readily produced by clinical state, environment, or other interventions; ***and*** Known pattern of response seen with other vaccines

7.5.2 Assessment of Severity

The severity of clinical and laboratory adverse events will be assessed according to the scales in **Tables 7-10**.

Table 7: Severity grading criteria for local adverse events

Adverse Event	Grade	Intensity
Pain at injection site	1	Pain that is easily tolerated
	2	Pain that interferes with daily activity
	3	Pain that prevents daily activity
Erythema at injection site*	1	>3 - ≤ 50mm
	2	>50 - ≤100mm
	3	>100mm
Swelling at injection site*	1	>3 - ≤ 20mm
	2	>20 - ≤ 50mm
	3	>50mm
Itching at injection site	1	Itching that is easily tolerated
	2	Itching that interferes with daily activity
	3	Itching that prevents daily activity

**** erythema or swelling ≤3mm is an expected consequence of skin puncture and will therefore not be considered an adverse event***

Table 8: Severity grading for physical observations

Adverse Event	Grade 1 (mild)	Grade 2 (moderate)	Grade 3 (severe)
Fever (oral)	37.6°C - 38°C	38.1°C - 39°C	>39°C
Tachycardia (bpm)*	101-115	116-130	>130
Bradycardia (bpm)**	50-54	40-49	<40
Systolic Hypertension (mmHg)	141-159	160-179	≥180
Diastolic Hypertension (mmHg)	91-99	100-109	≥110
Systolic Hypotension (mmHg)***	85-89	80-84	<80

****Taken after ≥10 minutes at rest***

***** When resting heart rate is between 60-100 beats per minute. Use clinical judgement when characterising bradycardia among some healthy subjection populations, for example, conditioned athletes.***

****** Only if symptomatic (e.g. dizzy / light headed)***

Table 9: Severity grading criteria for local and systemic AEs

Grade	Criteria
GRADE 0	None; Symptom not experienced
GRADE 1	Mild: Short-lived or mild symptoms; medication may be required. No limitation to usual activity
GRADE 2	Moderate: Mild to moderate limitation in activity. Medication may be required
GRADE 3	Severe: Considerable limitation in activity. Medication or medical attention required

Table 10: Severity grading criteria for laboratory AEs

Routine Haematology			Lab Range	Grade 0	Grade 1	Grade 2	Grade 3
Haemoglobin	**Male**	g/l	130 - 170	126 - 170	115 - 125	100 - 114	<100
	Female		120 - 150	114 - 150	103 - 113	90 - 104	<90
White blood cells	**Elevated**	$x10^9$/l	4.00 - 11.00	3.51 - 11.49	11.50 - 15.00	15.01 - 20.00	>20
	Low				2.50 - 3.50	1.50 - 2.49	<1.50
Platelets		$x10^9$/l	150 - 400	136 - 400	125 - 135	100 - 124	<100
Neutrophils		$x10^9$/l	2.0 - 7.5	1.5 - 7.5	1.00 - 1.49	0.50 - 0.99	<0.50
Lymphocytes		$x10^9$/l	1.5 - 4.0	1.0 - 4.0	0.75 - 0.99	0.50 - 0.74	<0.50
Eosinophils		$x10^9$/l	0.0 - 0.5	0.0 - 0.64	0.65 - 1.50	1.51 - 5.00	>5.00
Routine Biochemistry			**Lab Range**	**Grade 0**	**Grade 1**	**Grade 2**	**Grade 3**
Sodium	**Elevated**	mmol/l	133 - 146	133 - 146	147 - 148	149 - 150	>150
	Low				131 -132	129 - 130	<129
Potassium	**Elevated**	mmol/l	3.5 - 5.3	3.4 - 5.3	5.4 -5.5	5.6 - 5.7	>5.7
	Low				3.2 - 3.3	3.0 - 3.1	<3.0
Urea		mmol/l	2.5 - 7.8	2.5 - 8.1	8.2 - 8.9	9.0 - 11	>11
Creatinine		µmol/l	53 - 97	53 - 106	1.1-1.5*ULN	>1.5-3.0*ULN	>3.0*ULN
					107 - 145	146 -291	>291
Bilirubin	**Normal LFT**	µmol/l	0 - 20	0 - 25	1.3-1.5*ULN	>1.5-2.0*ULN	>2.0*ULN
					26 - 30	31 - 40	>40
Bilirubin[$]	**Abnormal LFT**	µmol/l	0 - 20	0 - 21	1.1-1.25*ULN	>1.25-1.5*ULN	>1.5-1.75*ULN
					22 - 25	26 - 30	>30
ALT/AST		IU/l			1.25-2.5*ULN	>2.5-5.0*ULN	>5.0*ULN
	Male		10 - 40	10 - 49	50 - 100	101 - 200	>200
	Female		7 - 35	7 - 43	44 - 87	88 - 175	>175
Alk Phosphatase		IU/l	30 - 130	30 - 142	1.1-2.0*ULN	>2.0-3.0*ULN	>3.0*ULN
					143 - 260	261 -390	>390
Albumin		g/l	35 - 50	32 - 50	28 - 31	25 - 27	<25

$ *When accompanied by any increase in LFT*

Coagulation studies	Lab Range	Grade 0	Grade 1	Grade 2	Grade 3
INR	0.8 - 1.2	0.8 - 1.2	1.3	1.4	≥1.5

7.6. Reporting procedures for AEs, SAEs and other safety events

7.6.1 Reporting Procedures for AEs

All AEs occurring in the 28 days following each vaccination observed by the Investigator or reported by the volunteer, whether or not attributed to study medication, will be recorded. AEs deemed to be not serious by the investigator will be recorded in the site's AE log and on the eCRF by the study team within 5 days of the study visit. AE data will be made available to the LSC before each interim safety analysis.

7.6.2 Reporting Procedures for SAEs

Serious adverse events (SAEs) will be collected throughout the entire trial period. 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 on the SAE forms by members of the study team immediately after the Investigators become aware of their occurrence. Copies of all reports will be forwarded for review to the Chief Investigator or delegated investigator and the Sponsor's representative (sponsor@uhs.nhs.uk) within 24 hours of the Investigator being aware of the suspected SAE. The local safety committee (LSC) will be notified of SAEs which are deemed possibly, probably or definitely related to study interventions; the LSC will be notified immediately (within 24 hours) of the Investigators' being aware of their occurrence by the Chief Investigator or delegated member of the study team. A copy of the SAE report will be sent to them for information and safety review purposes only; the LSC are not responsible for reporting of SAEs. SAEs will not normally be reported immediately to the REC 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 LSC. In addition to the expedited reporting above, the Investigator shall include all SAEs in the annual Development Safety Update Report (DSUR) report.

7.6.3 Reporting Procedures for SUSARS

The sponsor will report all SUSARs to the MHRA and REC within required timelines (15 days for all SUSARs, unless resulting in death or is life threatening in which case 7 days, with a final report within a further 8 days (total 15)). The Chief Investigator will also inform all Investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants.

All SUSARs and deaths occurring during the study will be reported to the Sponsor by the investigator by emailing sponsor@uhs.nhs.uk. For all deaths, available autopsy reports and relevant medical reports will be made available for reporting to the relevant authorities.

7.6.4 Development Safety Update Report

A Development Safety Update Report (DSUR) will be submitted by the Sponsor to the competent authority and ethics committee on the anniversary of the first approval date from the regulatory authority for the IMP.

7.6.5 Pregnancy Reporting

Volunteers that are pregnant or plan on becoming pregnant during the course of the study will not be enrolled.

Should a volunteer become pregnant during the trial, she will not receive any further investigational medicinal product and will be followed up as other volunteers and in addition will be followed until pregnancy outcome, with the volunteer's permission. We will not routinely perform venepuncture on such volunteers.

If a female subject or a female partner of a male subject becomes pregnant at any point during the trial, a completed trial specific Pregnancy Report must be submitted to the sponsor (sponsor@uhs.nhs.uk) within 1 business day of learning of its occurrence.

Figure 2: Safety Reporting Procedure

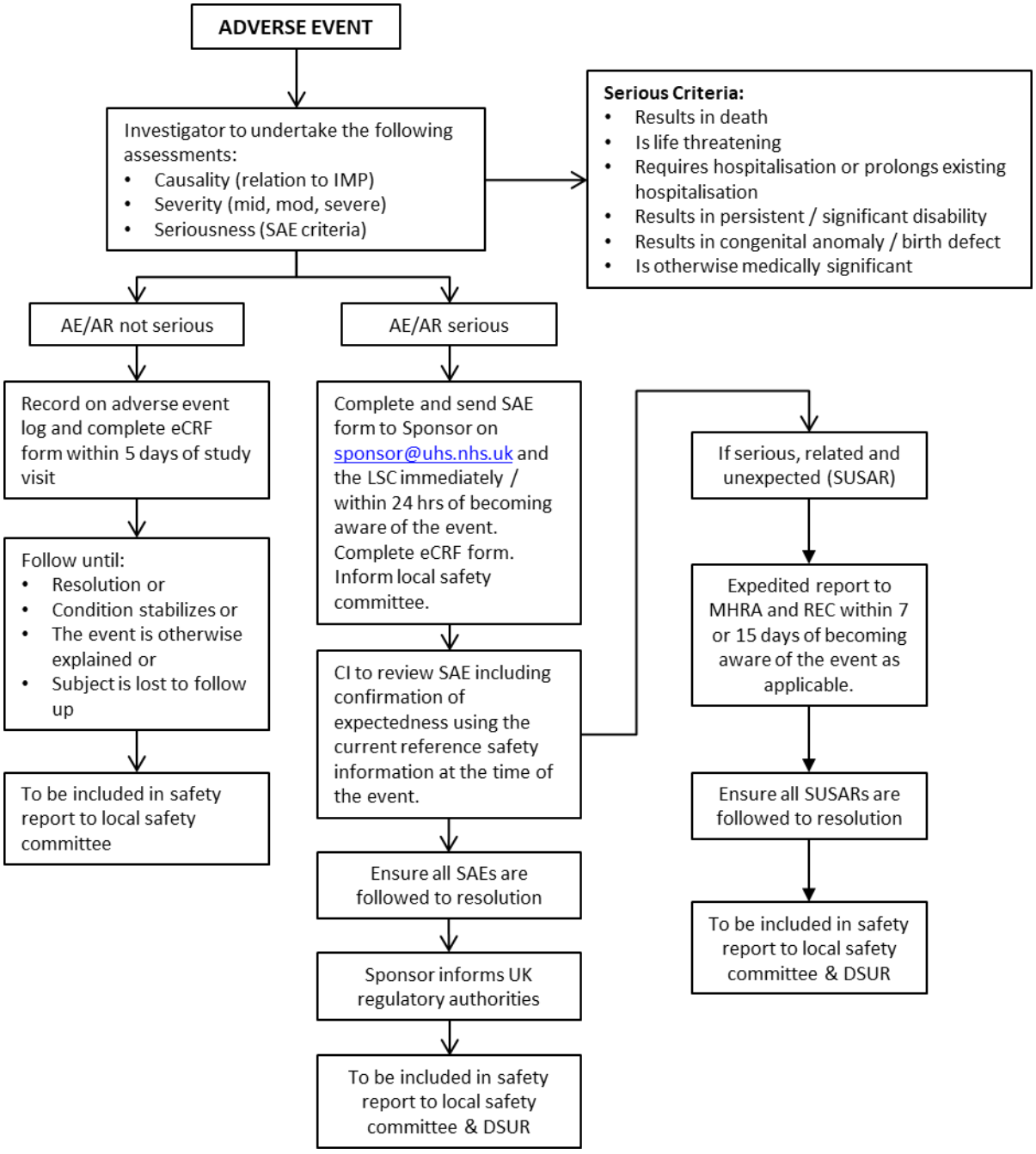


Table 11: Reporting periods for collecting safety information

	Screening	Day 0 Vaccine 1	Day 1 T/C	Day 3	Day 7	Day 14	Day 28 Vaccine 2	Day 42	Day 56	Day 84	Day 182	Day 365
Attendance number	1	2	3	4	5	6	7	8	9	10	11	12
Solicited local and systemic AEs												
Unsolicited AEs												
AEs/SAEs leading to withdrawal from the study												
SAEs (including adverse events of special interest)												
SAEs related to study participation (starting from provision of informed consent)												
Pregnancy (♀)												

7.7. Follow-up of AEs and SAEs

7.7.1. Follow-up during the study

In the event of AE's occurring during the study period 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.

7.7.2. Follow-up after the volunteer is discharged from the study

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. All AEs/SAEs leading to withdrawal from the study will be collected and recorded from the time of the first receipt of pEVAC-PS.

7.8. Procedures to be followed in the event of abnormal findings

Eligibility for enrolment in the trial in terms of laboratory findings will be assessed as detailed in Appendix A. Abnormal clinical findings from medical history, examination or blood tests will be assessed as to their clinical significance throughout the trial. Laboratory adverse events will be assessed using the details in Table 10. 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 and with the permission of the volunteer. Decisions to exclude the volunteer from enrolling in the trial or to withdraw a volunteer from the trial will be at the discretion of the Investigator.

7.9. Safety Stopping/ Holding Rules

Safety holding rules have been developed considering the fact that this is a first-in-human dose escalation study. Safety reviews will take place prior to each dose escalation as referenced in **Section 3.5**.

7.9.1. Group Holding Rules

The following holding rules will apply to all study groups. If a holding rule is activated, then further vaccinations will not occur until an internal safety review has been conducted by the LSC and it is deemed appropriate to restart dosing. All other procedures relating to safety and immunology will continue. The regulatory authority must be informed of the activation of any holding rule and a request to restart dosing with pertinent data must be submitted as a request for a substantial amendment. The internal safety review will consider:

- The relationship of the AE or SAE to the vaccine.

- The relationship of the AE or SAE to the vaccine dose, or other possible causes of the event.
- If appropriate, additional screening or laboratory testing for other volunteers to identify those who may develop similar symptoms and alterations to the current Participant Information Sheet (PIS) are discussed.
- New, relevant safety information from ongoing research programmes on the various components of the vaccine.

The Research Ethics Committee and IMP manufacturer will also be notified if a holding rule is activated or released. All vaccinated volunteers will be followed for safety until resolution or stabilisation (if determined to be chronic sequelae) of their AEs.

The group holding rules are as follows:

- One serious event considered by the investigator to be at least possibly related to the study drug
- Two serious adverse reactions considered by the investigator to be at least possible related to the study drug, irrespective of duration and whether the reactions are the same or not

These rules apply to both solicited and non-solicited adverse events as well as to laboratory adverse events.

7.9.2. Individual Stopping Rules

In addition to the above stated group holding rules, stopping rules will apply for individual volunteers in the occurrence of a serious adverse event considered by the investigator to be at least possibly related to the study drug. This applies to both solicited and unsolicited events as well as to laboratory parameters.

In addition to these pre-defined criteria, the study can be put on hold upon advice of the Chief Investigator, Study Sponsor, regulatory authority, Research Ethics Committee (REC) or Local Safety Committee (LSC), for any single event or combination of multiple events which, in their professional opinion, jeopardise the safety of the volunteers or the reliability of the data.

7.10. Local Safety Committee

A Local Safety Committee (LSC) will be appointed to provide real-time safety oversight. The LSC will review SAEs deemed possibly, probably or definitely related to study interventions. The LSC will be notified within 24 hours of the Investigators' being aware of their occurrence. The LSC has the power to place the study on hold if deemed necessary following a study intervention-related SAE. In addition to the LSC chair (LSM), there will be a minimum of two other appropriately qualified committee members. LSC membership details can be found in the *LSC Terms of Reference*. All correspondence between Investigator and LSC will be conveyed by the Investigator to the trial Sponsor.

The chair of the LSC 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.

The study can be put on hold upon advice of the Chief Investigator, Study Sponsor, REC or Local Safety Committee, for any single event or combination of multiple events which, in their professional opinion, jeopardise the safety of the subjects or the reliability of the data. If the study is placed on hold it may only be restarted following discussion with and approval from the LSC, the REC, the trial Sponsor and Chief Investigator. If the clinical trial is halted and is unable to resume, a formal letter will be sent to the regulatory authorities by the Sponsor explaining the reasons for cessation of the study.

7.11. Safety Profile Review

The safety profile will be assessed on an on-going basis by the Investigators. The Chief investigator, Principal Investigator, and relevant Investigators (as per the trial delegation log) will also review safety issues and SAEs as they arise.

7.12. Safety Reports and Dissemination Rules

Analyses presented to the local safety committee will be based on electronic case report form (eCRF) pages entered into the database. Additional data may be provided in narrative format by the study investigators of the study site. Data to be used for local safety committee review will be monitored prior to presentation to the committee using 100% source data verification. Information that will be included in safety reports and presented to the local safety committee at each meeting is documented in the *Local Safety Committee (LSC) Terms of Reference*

The local safety committee's decision, following review of the data presented, will be disseminated to the chief investigator, sponsor and key representatives of the trial management group. The signed safety report will be stored in the trial master file (TMF).

7.13. Emergency contact - 24 hours

Volunteers will be encouraged to contact one of the investigators on the 24 hour emergency mobile study telephone number if they develop any symptoms between the regular follow up visits. The investigator will consider an extra clinical review if the volunteer has any symptoms that are moderate or severe.

8. Statistics

A statistical analysis plan will be produced prior to patient enrolment. The final SAP will be signed off prior to final analyses at the end of trial.

8.1 Description of statistical methods

This is an open label, single-centre phase I study of the pEVAC-PS vaccine. The total number of volunteers enrolled in the study will be up to 36. If Group 3 is required to be opened up to participants, we will use the same dose as one of groups 2a or 2b (the most immunogenic and tolerated) so that 21 participants data will be available at that selected dose at the end of the study.

Due to the adaptive dose-finding procedure, we are seeking a dose level that is tolerable and yields as high a rate of reactogenicity in participants as possible. No formal hypothesis testing is being conducted on the primary outcomes and therefore no formal power calculations are necessary. Pragmatically, treating 6 patients in Group 1 is a larger cohort size than seen in many dose-finding studies (typically 1-3 patients are used). The more data acquired at this initial stage improves the chances of making the correct adaptation decision and end-of-trial recommendation. Further cohort sizes of 9 and 12 also provide sufficient numbers to make decisions and provided recommendations for larger phase II and III trials, whilst allowing for further adaptation if required later on in this study.

Dose-adaptation decisions will be made by the investigators using all available information on tolerability and immunogenicity at that timepoint (100% source data verification for all safety data, but not all immunogenicity will be available prior to the decision). Stopping rules will be followed as detailed in **Section 7.9.**

8.2 Statistical analyses

A statistical analysis plan will be produced prior to patient enrolment. This may affect the analyses that can subsequently be done – this will be documented in a revised statistical analysis plan.

Analysis of the safety and immunogenicity endpoints as detailed in **Section 2** will be performed primarily using descriptive statistics. Analysis will be as treated for safety endpoints and per protocol for immunogenicity.

The proportion of patients experiencing each adverse event observed, including biochemical and haematological samples outside the laboratory reference ranges, after each vaccine dose will be calculated and tabulated. For antibody measures means / medians, geometric means and geometric mean fold changes, with 95% confidence intervals will be calculated at each time point, Antibody data will also be described graphically through plotting titres at each time points for each group. The study is not powered for precise comparison of groups but for quantitative measures a non-parametric comparison will be done using either a Mann-Whitney

U test for between-group analyses or, for paired data between time points (e.g. baseline and post vaccination) using Wilcoxon matched pairs test (i.e. comparing numbers that increase vs decrease). If, on a log-scale antibody data are approximately normally distributed then comparison by t-tests and paired t-tests may also be done with adjustment for baseline levels if necessary.

8.3 Procedure for accounting for missing, unused and spurious data

Missing data will not be imputed. Patients with data missing on any variable required for a given analysis will be excluded from that analysis only. Outliers will be checked but not excluded if data errors are not found. Where results are reported as below or above assay limits a value of half or double the limit will be assigned for quantitative calculations. Antibody data will be logged for presentation or presented on log-scaled axis.

8.4 Procedures for reporting any deviations from the original statistical plan

These will be documented and reasons for changes given

9. Data Handling

9.1 Data collection tools and source document identification

9.1.1 Source Data

ICH E6 section 1.51, defines source data as "All information in original records and certified copies of original records or clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents (original records or certified copies)."

Source Data Verification (SDV) will be undertaken as part of the monitoring activities for the trial. See **Section 10.1** for further details.

9.1.2 Source Documents

Source documents include all original records of observations, results and activities necessary to reconstruct and evaluate the study. Source documents include but are not limited to laboratory reports, electrocardiogram tracings, volunteer diaries, hospital charts or pharmacy records, and any other records or reports of procedure performed during the study.

9.1.3 Case Report Forms (CRFs)

Patient data obtained during the study as a result of the study procedures, as well as relevant data from the medical history of the patient, findings as they occur, and other

relevant data must be reported to the sponsor by the investigator. Data must be reported in electronic Case Report Forms (eCRF) or other media approved by the sponsor. Different accounts are available for investigator site staff (Data Coordinator and Investigator).

An eCRF will be designed by an external vendor, Emmes, with assistance from the CI. The final eCRF will be approved by the sponsor. Site staff will be trained on completion and data entry requirements

9.2 Data Management

Data Management activities for the trial will be undertaken by an external vendor, Emmes India. This will include development of a data management plan, development, build and validation of the clinical trial database and coding of and reconciliation of data. At the end of the study and with sponsor approval, the database will be locked and export of data to the statistician will be undertaken. All Data Management activities will be undertaken in accordance with Emmes SOPs.

Primary data will be entered into the eCRF by delegated study team members. Primary sources of laboratory data will be from hospital computerised pathology results and, for clinical information, from clinical case notes or worksheets. Data queries will be raised via the eCRF and addressed by the site.

Participants will be informed during the consenting process that their data will be transferred outside of the EEA for the purposes of data management.

9.3. Access to data

The investigator(s)/ institution(s) will permit trial-related monitoring, audits, REC review and regulatory inspection(s) direct access to source data/documents. Trial participants are informed of this during the informed consent discussion. Participants will consent to provide access to their medical notes.

9.4. Data recording and record keeping

The investigators will maintain and retain appropriate medical and research records and essential documents for this trial in compliance with ICH E6 GCP and regulatory and institutional requirements for the protection of confidentiality of volunteers. The chief investigator, co-investigators and clinical research staff will have access to records. The investigators will permit authorised representatives of the sponsor, regulatory agencies and the monitors to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress.

9.5. Archiving

Archiving will be authorised by the Sponsor following submission of the end of study report.

Location and duration of record retention for:

- Essential documents: Patient case notes will be stored and maintained according to standard rules and procedures. Pathology results are stored and maintained according to standard procedures.
- The trial database: The trial database will be held by the DM vendor for a minimum period of 5 years from the completion of the study. A copy will be sent to the PI.

Destruction of essential documents will require authorisation from the Sponsor.

10. Quality Assurance

10.1 Study Monitoring

The monitoring activities for the trial will be undertaken by the CRO. A trial specific monitor will be assigned to the trial and a monitoring plan will be established and agreed by the sponsor. A risk-based approach to monitoring has been adopted for the trial. 100% source data verification (SDV) will take place for key study parameters and data used.

All monitoring reports will be sent to the sponsor for review following each monitoring activity. Monitoring reports are subject to review by the local safety monitor and local safety committee.

Auditing activities will be the responsibility of the Sponsor.

10.2 Audits and Statutory Inspections

The sponsor 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 Sponsor, trial site and regulatory authorities may carry out audit to ensure compliance with the protocol, GCP and appropriate regulations. GCP inspections may also be undertaken by the regulatory authority to ensure compliance with protocol and national regulations. The sponsor will assist in any inspections.

11. Ethical and Regulatory Consideration

The sponsor will ensure that the trial protocol, patient information sheet, consent form, GP letter and submitted supporting documents have been approved by the appropriate regulatory body (MHRA in UK), Health Research Authority (HRA), main research ethics committee (REC) and that local permission has been obtained prior to any participant recruitment. These activities have been delegated to the CRO for the purposes of this trial.

All substantial amendments (as determined by the sponsor) will not be implemented until HRA/REC and/or the MHRA have provided the relevant authorisations. The NHS R&D

departments will also be informed of any substantial amendments. Relevant approvals must be obtained before any substantial amendment may be implemented at sites.

All correspondence with the HRA, REC and the MHRA will be retained in the Trial Master File and the Investigator Site File (maintained by the site).

An annual progress report (APR) will be submitted to the REC (by the site) within 30 days of the anniversary date on which the favourable opinion was given, and annually until the trial is declared ended.

Within 90 days after the end of the trial (as defined in **section 5.8**), the CI/Sponsor will ensure that the HRA/ main REC and the MHRA are notified that the trial has finished. If the trial is terminated prematurely, those reports will be made within 15 days after the end of the trial.

The CI will supply the Sponsor with a summary report of the clinical trial, which will then be submitted to the MHRA and main REC within 1 year after the end of the trial.

All results will be published on a publicly accessible database (EUDRACT) in accordance with UK regulatory requirements.

11.1 Peer review

As part of the sponsorship approval process the protocol will be peer reviewed.

11.2 Public and Patient Involvement (PPI)

PPI contributors have been contacted to review the participant information sheet and for their contribution on an acceptable reimbursement for volunteer time.

11.3 Regulatory Compliance

The trial will not commence until a Clinical Trial Authorisation (CTA) is obtained from the MHRA, a favourable opinion obtained from the REC and HRA approval is obtained. Local capacity and capability will be obtained before any participant activity is undertaken.

For any amendment that will potentially affect a site's capacity and capability, the Principal Investigator(s) or designee will confirm with that site's R&D department that capacity and capability is ongoing.

The protocol and trial conduct will comply with the Medicines for Human Use (Clinical Trials) Regulations 2004/SI 1031 and any relevant amendments.

11.4 Protocol Compliance

The Investigator agrees to comply with the requirements of the Protocol and Good Clinical Practice. Prospective, planned deviations or waivers to the protocol are not allowed under the UK regulations on Clinical Trials and must not be used e.g. it is not acceptable to enrol a subject if they do not meet the eligibility criteria or restrictions specified in the trial protocol.

Accidental protocol deviations can happen at any time. They must be adequately documented on the relevant forms and reported to the Chief Investigator and Sponsor immediately.

Deviations from the protocol, which are found to frequently recur, are not acceptable and will require immediate action by the sponsor. Frequent non-compliances could potentially be classified as a serious breach (see section 11.5).

11.5 Notification of serious breaches to GCP and/ or the protocol

A "serious breach" is a breach which is likely to effect to a significant degree:

(a) the safety or physical or mental integrity of the subjects of the trial; or
(b) the scientific value of the trial

The sponsor will be notified immediately of any case where the above definition applies during the trial conduct phase and will notify the MHRA GCP Inspectorate using the following email address:
GCP.SeriousBreaches@mhra.gov.uk
and REC in writing of any serious breach of:

(a) the conditions and principles of GCP in connection with that trial; or
(b) the protocol relating to that trial, as amended from time to time
(c) within 7 days of becoming aware of that breach.

The MHRA template form for notifications of serious breaches to the MHRA will be used to ensure all appropriate information is submitted to the GCP Inspectorate.

11.6 Data protection and patient confidentiality

All investigators and trial site staff will comply with the requirements of the Data Protection Regulation with regards to the collection, storage, processing and disclosure of personal information and will uphold the Regulation's core principles.

The Case Report Forms (CRFs) will not contain subject names or other personal identifiable data. The subject's initials, date of birth and trial identification number, will be used for identification.

No subject identifiable data will be transferred to the sponsor, CRO or data management vendor.

The sites will hold a master subject list (with identifiable information) that will remain in the site file and on password protected computer systems only.

11.7 Financial and other competing interests for the chief investigator and committee members for the overall trial management

The CI (S Faust) has no competing interests relevant to this vaccine or trial. SF conducts commercial and non-commercial clinical trials of paediatric and adult vaccines and antimicrobial agents on behalf of University Hospital Southampton NHS Foundation Trust

and/or the University of Southampton. No committee members have relevant competing interests to be declared at the time of protocol writing.

11.8 Indemnity

The NHS Negligence Scheme will apply for this study.

11.9 Amendments

Under the Medicines for Human Use (Clinical Trials) Regulations 2004, the sponsor may make a non-substantial amendment at any time during a trial. If the sponsor wishes to make a substantial amendment to the CTA or the documents that supported the original application for the CTA, the sponsor must submit a valid notice of amendment to the licensing authority (MHRA) for consideration. If the sponsor wishes to make a substantial amendment to the REC application or the supporting documents, the sponsor must submit a valid notice of amendment to the REC for consideration. The MHRA and/or the REC will provide a response regarding the amendment within 35 days of receipt of the notice. It is the sponsor's responsibility to decide whether an amendment is substantial or non-substantial for the purposes of submission to the MHRA and/or REC.

Amendments need to be notified to NHS R&D departments of participating sites to assess whether the amendment affects the capacity and capability for that site.
All amendments must be notified to the HRA and CRN for categorisation. Implementation will be based upon the amendment category assigned.

Version control will be maintained for any amendments made. Substantial Amendments (SAs) will be identified by a whole number change (i.e. v1.0, v2.0) and non-substantial amendments (NSAs) identified by a decimal change (i.e. v1.1, v1.2). All amendments will be logged at each site and a central copy maintained in the TMF.

Guidance on the categorisation of amendments can be found on the HRA website http://www.hra.nhs.uk/resources/after-you-apply/amendments/

12. Dissemination Policy

On completion of the study a final check on the data will be performed and any missing data collected or otherwise accounted for. Data will be tabulated and analysed as described in the statistical plan. A final study report will be prepared by the CI for submission to the REC, MHRA, Sponsor and R&D. The time period from the completion of follow-up of the last trial subject and submission of the report will follow regulatory guidelines. All publications relating to the study will follow Consort Guidelines.

It is anticipated that the study will be prepared for submission to meetings and for publication. Any publication must have the approval of the CI/PI. There will be no time limit for the production of any publication.

Participants in the study will be informed of the final results. Participants will be informed following the preparation of the final report and may be provided with a lay person's summary of the conclusions.

12.1 Authorship eligibility guidelines and any intended use of professional writers

The list of authors will consist of those individuals who, at the discretion of the CI, have contributed to the creation of the study itself, to the recruitment and care of subjects, to the collection and analysis of the data, to the preparation of any reports, abstracts and commentary and final publication of the study.

13. References

FDA. Toxicity Grading Scale for Healthy Adult & Adolescent Volunteers enrolled in Preventative Vaccine Clinical Trials. 2007.

14. Appendices

Appendix A: Laboratory Values for Exclusion

Laboratory parameters for inclusion/exclusion in the trial will be considered on an individual basis, with investigator discretion for interpretation of results and the need for repeated or further tests. In general, volunteers will be excluded if a result at screening constitutes what would qualify as a grade 1 (or higher) laboratory adverse event, according to the site-specific laboratory adverse event tables (filed in the TMF). Urinalysis at screening will be assessed as per the table below:

URINE ANALYSIS (using MULTISTIX)	
Protein*	2+ or Protein creatinine ratio of ≥50mg/mmol
Blood£	2+ on two dipstick tests
Glucose	1+

**In the event of the dipstick testing positive for protein with ≥1+ protein urine should be sent for a protein creatinine ratio.*

£In the event of urine dipstick testing positive for ≥1+ blood with, or without, protein in volunteers a repeat dipstick test will be carried out to confirm haematuria. In female volunteers, a menstrual history will be taken to elicit whether the subject is currently menstruating and if they are, urine dipstick will be repeated after 1 - 2 weeks. If blood and/or proteinuria persist in any volunteer, an interpretation of the results will be undertaken by the investigator on an individual basis to determine if they will be excluded from the trial, and the appropriate follow-up arranged.

Appendix B: Modification History

Version	Date	Author(s)	Modification