STUDY PROTOCOL

STUDY TITLE:	A Phase I, randomised, double-blind, placebo-controlled, parallel group dose escalation study to evaluate the safety, tolerability and immunogenicity of three doses of a potential oral enteric fever vaccine (ZH9 + ZH9PA) in healthy participants 18 to 45 years of age inclusive.
STUDY NUMBER:	PRK-ENT-001 RD760/34119
EudraCT NUMBER:	2019-002047-22
IRAS ID:	266061
INVESTIGATIONAL MEDICINAL PRODUCT (IMP)(s):	ZH9PA ZH9/ZH9PA Placebo
PLANNED STUDY DOSES:	3 doses of $1x10^9$ CFU of ZH9PA 3 doses of $1x10^{10}$ CFU of ZH9PA 3 doses of $1x10^{10}$ CFU of ZH9PA and $1x10^{10}$ CFU of ZH9 3 doses of placebo
PRINCIPAL INVESTIGATOR:	[redacted] Simbec-Orion Clinical Pharmacology, Merthyr Tydfil Industrial Park, Pentrebach, Merthyr Tydfil, CF48 4DR, United Kingdom (UK)
STUDY SPONSOR:	Prokarium Ltd. London Bioscience Innovation Centre, 2 Royal College St, London, NW1 0NH, UK
STUDY SPONSOR'S RESPONSIBLE PHYSICIAN:	[redacted]

STUDY MONITOR: [redacted]

ADDITIONAL DEPARTMENTS: [redacted]

PROTOCOL FINALISATION STATEMENT

This protocol is not considered final unless accompanied by a letter of favourable opinion from the Research Ethics Committees and Notice of Acceptance from the relevant Competent Authority.

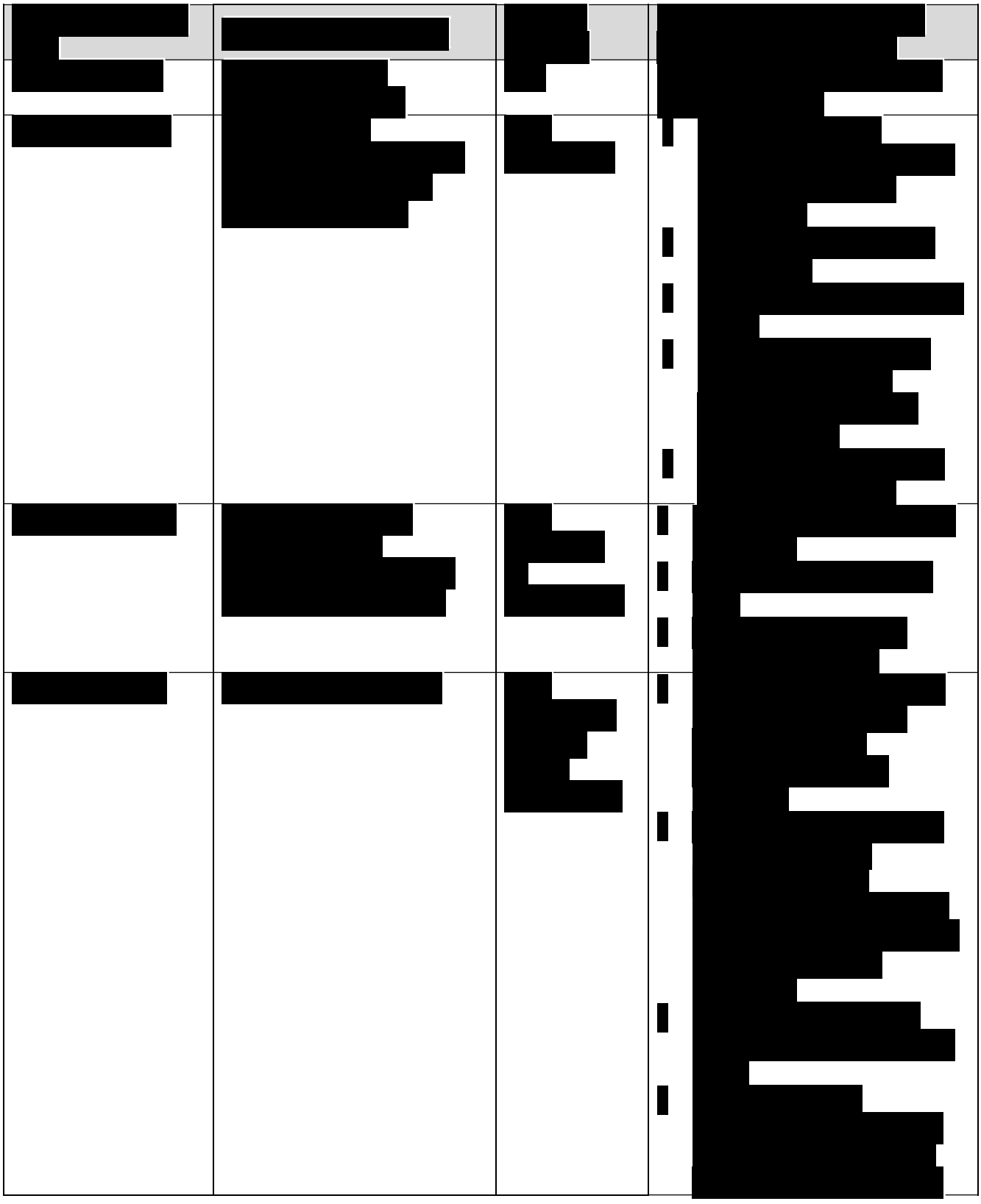
Protocol Prepared by: LL

1 SIGNATURE PAGE

Prokarium Ltd (the Sponsor) has reviewed and approves the contents of this protocol, PRK-ENT-001 and consider it appropriate to administer the vaccine to healthy adult participants in accordance with the protocol. Prokarium agrees that it will arrange for the supply of the investigational products described in the protocol and undertakes to report serious adverse events and serious breaches of GCP to the relevant authorities in compliance with the regulations. It further agrees to inform the Investigators of any information that would place the participants at risk by their continuing participation in the trial.

I declare that I have read and understood this study protocol. I agree to abide by this protocol (subject to any amendments agreed in writing between the Sponsor and Principal Investigator). Any changes in procedure will only be made if necessary, to protect the safety, rights or welfare of the participants.

2 PROTOCOL AMENDMENT/REVISION HISTORY



			[redacted]

3 SYNOPSIS

NAME OF COMPANY: Prokarium Ltd.

NAME OF INVESTIGATIONAL MEDICINAL PRODUCT: ZH9PA and ZH9/ZH9PA

NAME OF ACTIVE INGREDIENT: ZH9 and ZH9PA

TITLE OF STUDY: A Phase I, randomised, double-blind, placebo-controlled, parallel group dose escalation study to evaluate the safety, tolerability and immunogenicity of three doses of a potential oral enteric fever vaccine (ZH9 + ZH9PA) in healthy participants 18 to 45 years of age inclusive.

PRINCIPAL INVESTIGATOR: [redacted]

STUDY CENTRE: Simbec-Orion Clinical Pharmacology,
Merthyr Tydfil, CF48 4DR, UK

CLINICAL PHASE: I

OBJECTIVES:

Primary Objective

- To evaluate the safety and tolerability of three doses of two dose levels of ZH9PA and of three doses of a single dose level of a combination of ZH9 and ZH9PA over 12 weeks (up to Day 84) after the first dose of vaccine.

Secondary Objectives

- To evaluate serum immunoglobulin (Ig) A and IgG responses against the following antigens: - *S. enterica* Ser. Paratyphi A lipopolysaccharide (LPS) 0:2 and Flagella H:a and *S. enterica* ser. Typhi LPS O:9 and Flagella H:d, induced by three vaccine doses, of two dose levels ($1x10^9$ colony forming units (CFU) and $1x10^{10}$ CFU) of ZH9PA alone or in combination with $1x10^{10}$ CFU ZH9, up to Day 84 after the first dose of vaccine (Day 0).

- To evaluate the mucosal IgA immune responses to LPS 0:2, LPS O:9, Flagella H:a and Flagella H:d, at 7 days after each vaccination, as determined by antibodies in lymphocyte supernatant (ALS) assay.

- To evaluate Serum IgA and IgG responses against the four antigens, induced by each of the first two vaccine doses, on Days 21, and 42, by blood samples taken prior to each participant receiving the next dose of vaccine or placebo.

- To evaluate the safety profile of the ZH9PA and combination ZH9/ZH9PA vaccines, over the 6 months following the third dose of vaccine.

METHODOLOGY:

This is a Phase I, randomised, double-blind, placebo-controlled, parallel group, single-centre study involving 45 healthy participants. The aim is to evaluate a combination vaccine against enteric fever comprising the live attenuated Salmonella strain ZH9 (previously tested in a number of clinical trials as a typhoid vaccine) and its modified derivative, ZH9PA, in which the typhoid antigens LPS O:9 and Flagellin H:d have been substituted by the Paratyphi A antigens, LPS O:2 and Flagellin H:a. ZH9PA has not previously been tested in humans; therefore, the first two cohorts comprise a dose escalation of this component alone, followed by a final cohort in which the combination is tested at a single dose level. The study will remain fully blinded until Day 70, when a stool sample is taken to assess if participants are shedding the bacterial strains of the vaccine in their faeces. The Investigators and the Monitor may be unblinded to the treatment allocation of participants who have a stool culture positive for any *Salmonella* strain, as it will be presumed to be one of the vaccine strains, and the participant will receive a course of antibiotics.

NUMBER OF PARTICIPANTS: 45 healthy participants 18 to 45 years of age, inclusive.

INCLUSION CRITERIA:

To be confirmed at Screening

1. Healthy male and female participants 18 to 45 years of age, inclusive.
2. Female participant of childbearing potential willing to use 2 effective methods of contraception, i.e., established method of contraception + condom, if applicable (unless of non-childbearing potential or where abstaining from sexual intercourse is in line with the preferred and usual lifestyle of the participant) from the first dose until 2 months after the last dose of IMP.
3. Female participant of non-childbearing potential. *For the purposes of this study, this is defined as the participant being amenorrhoeic for at least 12 consecutive months or at least 4 months post-surgical sterilisation (including bilateral fallopian tube ligation or bilateral oophorectomy with or without hysterectomy).*
4. Female participant of childbearing potential or non-childbearing potential with a negative pregnancy test at Screening.
5. Female participant of post-menopausal status confirmed by demonstrating at Screening that the serum level of the follicle stimulating hormone (FSH) falls within the respective pathology reference range. In the event a participant's menopausal status has been clearly established (for example, the participant indicates she has been amenorrhoeic for 10 years, confirmed by medical history, etc), but serum FSH levels are not consistent with a postmenopausal status, determination of the participant's eligibility to be included in the study will be at the Investigator's discretion following consultation with the Sponsor.
6. Male participant willing to use an effective method of contraception or 2 effective methods of contraception, i.e., established method of contraception + condom, if applicable (unless anatomically sterile or where abstaining from sexual intercourse is in line with the preferred and usual lifestyle of the participant) from first dose until a stool sample tested for presence of the vaccine strains is negative.
7. Participant with a body mass index (BMI) of ≥ 19 or ≤ 34 kg/m^2 (BMI = body weight (kg) / [height (m)]^2).
8. No clinically significant history of liver or active gall bladder disease.
9. No clinically significant history of ongoing gastro-intestinal disease or abnormality.
10. No clinically significant history of previous allergy / sensitivity to ZH9/ZH9PA or sodium bicarbonate.
11. No clinically significant history of anaphylactic shock following vaccination.
12. No clinically significant history of hypersensitivity (e.g., hives/rash/swollen lips/difficulty with breathing) to azithromycin, ampicillin, trimethoprim-sulfamethoxazole or ciprofloxacin.
13. No clinically significant abnormal laboratory test results (in the opinion of the investigator) for serum biochemistry, haematology and/or urine analyses within 28 days before receiving the first dose administration of the IMP.
14. Participant with a negative urinary drugs of abuse (DOA) screen (including alcohol and cotinine) test results, determined within 28 days before the first dose administration of the IMP unless there is a documented medical explanation for the positive result other than drugs of abuse (e.g., the participant has been prescribed opioids for pain). (N.B.: A positive test result may be repeated at the Investigator's discretion).
15. Participant with negative human immunodeficiency virus (HIV), hepatitis B surface antigen (HBsAg)) and hepatitis C virus antibody (HCV Ab) test results at Screening.
16. No clinically significant abnormalities in 12-lead electrocardiogram (ECG) or vital signs determined within 28 days before first dose of IMP.

17. Participant must be available to complete the study (including all follow up visits).
18. Participant must be willing to consent to have data entered into The Over Volunteering Prevention System (TOPS).
19. Participant must provide written informed consent to participate in the study.

To be re-confirmed on Day 0 / prior to each dosing visit

1. Participant continues to meet all screening inclusion criteria.
2. Participant with a negative urinary drugs of abuse screen (including alcohol and cotinine) prior to dosing unless there is a documented medical explanation for the positive result other than drugs of abuse (e.g., the participant has been prescribed opioids for pain). (N.B.: A positive test result may be repeated at the Investigator's discretion).
3. Female participant of childbearing potential or non-childbearing potential with a negative pregnancy test on admission.

EXCLUSION CRITERIA:

To be confirmed at Screening:

1. Participant with any clinically significant medical (cardiovascular disease, pulmonary, hepatic, gallbladder or biliary tract, renal, haematological, gastrointestinal, endocrine, immunologic, dermatological, neurological, autoimmune disease or current infection) or psychiatric condition (see also exclusion criterion number 214) that, in the opinion of the Investigator, precludes participation in the study. This will include any clinically significant abnormal serum biochemistry results and/or haematological results and/or urine analytical results.
2. Participant with a history of heart disease or of rheumatic fever.
3. Participant with a significant acute febrile illness (including fever of 38.0°C or greater within 14 days) of each dose of IMP (Days 0, 21 and 42).
4. Participant who has chronic diseases: Chronic diseases will include all autoimmune and immunocompromising conditions and any other chronic condition, which at the judgment of the Investigator, may put the participant at higher risk of side effects from the study vaccine. Conditions in the latter category might include unexplained anaemia, hepato-biliary disease, uncontrolled hypertension, participant with prosthetic joints or heart valves, etc.
5. Participant with sickle cell anaemia.
6. Participant who has undertaken a course of antibiotics/antibacterials within 28 days prior to each dose of IMP (Days 0, 21 and 42).
7. Use of prescription or non-prescription drugs within 28 days or 5 half-lives (whichever is longer) prior to receiving the first dose of IMP, unless in the opinion of the Investigator and Sponsor's Responsible Physician the medication will not interfere with the study procedures or compromise participant safety.
8. Participant who uses antacids, proton pump inhibitors or H_2 blockers on a regular basis or has consumed proton pump inhibitors or H_2 blockers within 24 hours prior to each dose of IMP.
9. Participant who has received investigational or licensed vaccines in the 28 days prior to dosing or anticipates receiving a vaccine other than study medication up to Day 84 of the study.
10. Participant with symptoms consistent with Typhoid fever concurrent with travel to countries where typhoid infection is endemic (most of the developing world) within 2 years prior to first dose of IMP.
11. Vaccination against Typhoid within 3 years prior to first dose of IMP.
12. Ingestion of Typhoid bacteria in a challenge study within 3 years prior to dosing.

13. Participant who works as a commercial food handler.
14. Participant who is a health care worker in direct contact with patients.
15. Participant who is a childcare worker.
16. Participant who has household contact with immuno-compromised individuals, pregnant women, children < 2 years of age or individuals > 70 years of age.
17. Participant who has person(s) living with him/her who, in the opinion of the Investigator, may be at risk of disease if exposed to the vaccine strain.
18. Participant with a known impairment of immune function or receiving (or has received in the 6 months prior to study entry) cytotoxic drugs or immunosuppressive therapy (including systemic corticosteroids).
19. Participant who is a current smoker (cigarettes, tobacco and/or e-cigarettes) or has stopped smoking in the last 3 months prior to Screening.
20. A clinically significant history of drug or alcohol abuse [defined as the consumption of more than 14 units of alcohol a week] within the past two years prior to Screening.
21. Inability to communicate well with the Investigators (i.e., language problem, poor mental development or impaired cerebral function).
22. Participation in a New Chemical Entity (NCE) clinical study within the previous 3 months or a marketed drug clinical study within the 30 days before the first dose of IMP. *(Washout period between studies is defined as the period of time elapsed between receiving the last dose of the previous study and receiving the first dose of the next study).*
23. Donation of 450 millilitres (mL) or more blood within the 3 months before the first dose of IMP.
24. Participant who, in the opinion of the Investigator, is unsuitable for participation in the study.

To be re-confirmed at Day 0 / prior to each dosing visit:

1. Development of any exclusion criteria since the Screening visit.
2. Use of prescription or non-prescription drugs since Screening, unless in the opinion of the Investigator and Sponsor's Responsible Physician, the medication will not interfere with the study procedures or compromise participant safety.
3. Participation in a clinical study since Screening.
4. Donation of 450 mL or more blood since Screening.

IMP ADMINISTRATION:

Cohort 1 will consist of 9 participants, who will receive 3 doses of $1x10^9$ CFU of ZH9PA (6 participants) or placebo (3 participants).

Cohort 2 will consist of 18 participants, who will receive 3 doses of $1x10^{10}$ CFU of ZH9PA (12 participants) or placebo (6 participants).

Cohort 3 will consist of 18 participants, who will receive 3 doses of $1x10^{10}$ CFU of ZH9PA and $1x10^{10}$ CFU of ZH9 (12 participants) or placebo (6 participants).

CRITERIA FOR EVALUATION:

Primary Endpoint:

- Local and systemic reactogenicity up to Day 84 as assessed by adverse events, laboratory safety tests (biochemistry, haematology, urinalysis), vital signs and physical examination.

Secondary Endpoints:

- Local and systemic reactogenicity; from Day 85 to Day 224 as assessed by adverse events, laboratory safety tests (biochemistry, haematology, urinalysis) if undertaken for cause; vital signs if undertaken for cause.

- Concentrations of specific serum IgA and IgG antibodies to LPS O:2 and O:9, flagellin H:a and H:d.
- Fold increase in specific serum IgA and IgG antibody concentrations, in individual participants, against the antigens LPS O:2 and O:9, flagellin H:a and H:d.
- Concentrations of specific mucosal IgA ALS assays, in individual participants, against antigens LPS O:2 and O:9, flagellin H:a and H:d.
- Seroconversion rate (proportion of participants with 4-fold increase above baseline at any time post vaccination) against each of the above antigens.
- Fold increase in specific mucosal IgA ALS assays, in individual participants, against the antigens LPS O:2 and O:9, flagellin H:a and H:d.

STATISTICAL METHODS: All statistical analysis will be performed using SAS® (version 9.3 or higher).

Descriptive statistics for quantitative parameters will be provided using number of observations (N), mean, standard deviation (SD), minimum, median and maximum. Descriptive statistics for qualitative parameters will be provided using absolute frequencies (n) and relative frequencies (%). For immunological endpoints (IgG, IgA, concentrations and fold increase) additionally geometric means with corresponding 95% confidence intervals (CI) will be presented.

For parameters with evaluation before vaccination and in case of repeated value(s), only the last observation prior to dosing will be used in descriptive and inferential statistics and derivations of other parameter values. After vaccination, only values of scheduled assessments (planned in the protocol) will be used.

The sample size chosen for this study is not based on a formal statistical estimation but is considered to be adequate to meet the objectives of the study. A sufficient number of participants will be initially screened for enrolment to ensure that the planned sample size is achieved.

DURATION OF STUDY:	Approximately 36 weeks for each individual (from Screening to Post-Study Follow-Up).

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TABLES

5 ABBREVIATIONS USED IN THE PROTOCOL TEXT

- ABPI — Association of the British Pharmaceutical Industry
- AE(s) — adverse event(s)
- ALS — antibodies in lymphocyte supernatant
- BMI — body mass index
- bpm — beat(s) per min
- CFU — colony forming units
- CI — confidence interval
- cm — centimetre
- CTA — Clinical Trial Authorisation
- DOA — drugs of abuse
- eCRF — electronic case report form
- ECG — electrocardiogram
- ELISA — enzyme-linked immunosorbent assay
- EU — European Union
- FSH — follicle stimulating hormone
- g — gramme(s)
- GALT — gut-associated lymphoid tissue
- GCP — Good Clinical Practice
- GDPR — General Data Protection Regulation
- GMP — Good Manufacturing Practice
- HBsAg — hepatitis B surface antigen
- HCV Ab — hepatitis C virus antibody
- HIV — human immunodeficiency virus
- IB — Investigator's Brochure
- ICH — International Council on Harmonisation
- Ig — immunoglobulin
- IMP — investigational medicinal product(s)
- ISF — investigator site file
- kg — kilogram
- LMICs — low- and middle-income countries
- LPS — lipopolysaccharide
- Ltd — Limited
- MedDRA — Medical Dictionary for Regulatory Activities
- MHRA — Medicines and Healthcare products Regulatory Agency
- mL — millilitre
- min(s) — min(s)
- NCE — new chemical entity
- NSAID — non-steroidal anti-inflammatory drug
- PBMC — peripheral blood mononuclear cells
- PD — pharmacodynamic
- PR interval — time the electrical impulse takes to travel from the sinus node through the AV node and entering the ventricles; measured from the beginning of the P wave to the beginning of the QRS complex
- QA — quality assurance
- QP — qualified person
- QRS — QRS complex represents ventricular depolarisation
- QT interval — the time for both ventricular depolarisation and repolarisation to occur, and therefore roughly estimates the duration of an average ventricular action potential.
- QTc — corrected QT interval
- REC — Research Ethics Committee
- SAE — serious adverse event
- SAP — statistical analysis plan
- SAR — serious adverse reaction
- SAS — statistical analysis software by SAS Institute Inc., USA
- SD — standard deviation
- Ser. — serovar
- SHM — sample handling manual
- SmPC — Summary of Product Characteristics

- SOC system organ class
- SOP standard operating procedure(s)
- SPI2 salmonella pathogenicity island 2
- SUSAR suspected unexpected serious adverse reaction
- TEAE treatment emergent adverse event
- TOPS The Over Volunteering Prevention System
- UK United Kingdom
- VMF volunteer master file
- WBC white blood cell(s)
- w/v weight/volume

6 ETHICS

6.1 Research Ethics Committee or Institutional Review Board

This study protocol will be submitted to the Research Ethics Committee (REC) for review to confirm provision of a favourable opinion. The favourable opinion of the REC must be obtained before commencement of any study procedures.

The favourable opinion is conditional upon the Sponsor registering the clinical trial in a publicly accessible database, within 6 weeks of the first participant recruited.

All substantial protocol amendments must receive favourable opinion from the REC responsible for the study. Non-substantial amendments will not require favourable opinion from the REC.

If the study is stopped due to adverse events (AEs) it will not be recommenced without reference to the REC responsible for the study.

The outcome of the study (e.g., completed) will be reported to the REC responsible for the study within 90 days of completion of the last participant's final study procedures. In the event of the study being prematurely terminated a report will be submitted to the REC responsible for the study within 15 days.

A summary of the clinical study report will be submitted to the REC responsible for the study within 1 year of completion of the last participant's final study procedures.

The REC will be informed that Simbec-Orion is a commercial organisation and that the study is funded by Prokarium Ltd. The participants who take part in the clinical study will be paid for their inconvenience and have been informed that there will be no benefits gained by their participation. All potential conflicts of interest will be declared by the Investigators.

6.2 Ethical Conduct of the Study

The Principal Investigator shall be responsible for ensuring that the clinical study is performed in accordance with the following:

- Declaration of Helsinki (Brazil, 2013)[1] (Appendix 1).
- Association of the British Pharmaceutical Industry (ABPI) Guidelines for Phase 1 Trials (2018)[2].
- ICH (International Council on Harmonisation) Guideline for Good Clinical Practice (GCP) E6 (R2) (CPMP/ICH/135/95) 1996[3].
- The Medicines for Human Use (Clinical Trials) Regulations 2004 (Statutory Instrument 2004 No. 1031) and subsequent amendments[4].

This clinical study has been registered in the EudraCT database and a Clinical Trials Authorisation (CTA) will be obtained from the Medicines and Healthcare products Regulatory Agency (MHRA) prior to the start of the study in accordance with Part 3, Regulation 12 of the UK Statutory Instrument.

6.3 Participant Information and Consent

Potential participants who volunteer for participation in the study will be informed of the aims, methods, anticipated benefits and potential hazards of the study and any possible discomfort it may entail. Information will be given in both oral and written form and in the manner deemed appropriate by the Clinical Unit standard operating procedures (SOPs). Each participant will also be informed of his/her right to withdraw from the study at any time, for any reason.

A written explanation (participant information sheet) and informed consent form will be provided, and the participant will be allowed sufficient time to consider the study information. Prior to signing the informed consent form, the participant will be given an opportunity to discuss any issues concerning the study with an Investigator who has suitable knowledge of the study and will have all questions answered openly and honestly.

If the participant is willing to participate in the study, the informed consent form will be signed and personally dated by the participant and the person taking consent. The participant will receive a copy of the informed consent form together with the participant information sheet and the original signed informed consent form will be retained with the study records at the Investigator site. In addition, the actions and completion of the consenting process will be recorded in the participant's medical record (i.e., source document).

7 INVESTIGATORS AND STUDY ADMINISTRATIVE STRUCTURE

The study will be performed at a single site, Simbec-Orion Clinical Pharmacology Unit. [redacted]
The Project Manager will act on behalf of the Principal Investigator to ensure the smooth and efficient running of all aspects of the study.

7.1 Study Personnel

[redacted]

7.2 Indemnity Arrangements

[redacted]

8 INTRODUCTION

Prokarium is developing a bivalent vaccine against Enteric Fever, caused by infection with typhoidal serovars, namely *Salmonella enterica* serovar (ser.) Typhi and *Salmonella enterica* ser. Paratyphi A. These serovars are human-adapted bacterial pathogens that cause related, potentially fatal, systemic diseases, collectively called enteric fever or typhoid; the name being derived from the ancient Greek typhos, an ethereal smoke or cloud that was believed to cause disease and madness. The infectious reservoir for the bacteria is patients with an acute infection that shed the bacteria in their stools and chronic carriers, patients who have a reservoir of bacteria, usually in their gall bladder, who periodically shed viable bacteria in their stools. The classic presenting symptoms include fever, malaise, diffuse abdominal pain, and constipation although approximately one third of patients develop diarrhoea after the occurrence of the fever[6]. Left untreated, enteric fever can progress to delirium, intestinal haemorrhage and perforation of the bowel and ultimately death. *Salmonella enterica* ser. Typhi and *Salmonella enterica* ser. Paratyphi A have been eradicated from developed countries but thrive in areas of poor sanitation and remain a significant health problem in low- and middle-income countries (LMICs) of Southeast Asia, the Indian subcontinent, Africa, and, to a lesser extent, South America[7]. It has been estimated that the total number of typhoid fever episodes in 2010 was 13.5 million (interquartile range 9.1-17.8 million). The adjusted estimate accounting for the low sensitivity of blood cultures for isolation of the bacteria was 26.9 million (interquartile range 18.3-35.7 million) episodes[8].

The disease is spread through the ingestion of contaminated food and water. *S. enterica* Typhi and *S. enterica* ser. Paratyphi A are highly invasive and adapted to avoid the innate immune system. Once ingested, the bacteria pass through the intestinal mucosa, mainly of the distal ileum. The bacteria have specialised fimbriae that adhere to the epithelium over the lymphoid tissue of the Peyer's patches in the ileum. The bacteria are taken up by the macrophages and pass into the lymphatic system and then induce their host macrophages to attract more macrophages to the gut-associated lymphoid tissue (GALT). The *Salmonella* reproduce within the macrophages, as they are carried through the mesenteric lymph nodes to the thoracic duct and the lymphatics, and then through to the reticuloendothelial tissues of the liver, spleen, bone marrow and lymph nodes. Once in the reticuloendothelial system, multiplication continues over a period of 8 to 14 days until apoptosis of the macrophages results in a bacteraemia and systemic spread. The bacteria then infect the gallbladder via either bacteraemia or direct extension of infected bile. The result is that the organism re-enters the gastrointestinal tract in the bile and re-infects Peyer patches. Bacteria that do not re-infect the host are typically shed in the stool and are then available to infect other hosts.

[redacted]

[redacted] the widespread use of antibiotics in developing countries is leading to the development of resistance in typhoidal serovars. Hence, there is a need for a vaccine to protect patients against antibiotic resistant strains of bacteria[7].

ZH9 has previously been administered to humans at a maximum dose of 1.7 x 10^{10} CFU and been shown to be well tolerated and immunogenic. It is anticipated that a similar dose of ZH9PA will be required. It is intended that the vaccine contains equal amounts of ZH9 and ZH9PA and therefore, this study will explore two dose levels of ZH9PA and a single dose level of ZH9 plus ZH9PA given in combination. Previous work with ZH9 has identified that a single dose does not give optimal protection against a typhoid challenge strain but it was demonstrated a single dose provided a significant reduction in the microbiological burden of infection and alteration of the clinical disease profiles[12]. Hence, the safety and immunogenicity of three doses, administered 3 weeks apart will be investigated in this study.

Further details of the non-clinical studies and a summary of the known and potential risks and benefits to human participants of ZH9 and ZH9PA can be found in the IB[11].

The study will be conducted in compliance with the protocol, GCP and the applicable regulatory requirement(s), as indicated within Section 6.2.5 of the ICH GCP E6 (R2) guidelines[3].

9 STUDY OBJECTIVES

9.1 Primary Study Objective

The primary objective is to evaluate the safety and tolerability of three doses of two dose levels of ZH9PA and of three doses of a single dose level of a combination of ZH9 and ZH9PA over 12 weeks (up to Day 84) after the first dose of vaccine.

9.2 Secondary Study Objective

- To evaluate serum IgA and IgG responses against the following antigens: - *S. enterica* Ser. Paratyphi A LPS 0:2 and Flagella H:a and *S. enterica* ser. Typhi LPS O:9 and Flagella H:d, induced by three vaccine doses, of two dose levels ($1x10^9$ CFU and $1x10^{10}$ CFU) of ZH9PA alone or in combination with $1x10^{10}$ CFU ZH9, up to Day 84 after the first dose of vaccine (Day 0).

- To evaluate the mucosal IgA immune responses to LPS 0:2, LPS O:9, Flagella H:a and Flagella H:d, at 7 days after each vaccination, as determined by ALS assay.

- To evaluate Serum IgA and IgG responses against the four antigens, induced by each of the first two vaccine doses, on Days 21, and 42, by blood samples taken prior to each participant receiving the next dose of vaccine or placebo.

- To evaluate the safety profile of the ZH9PA and combination ZH9/ZH9PA vaccines, over the 6 months following the third dose of vaccine.

10 INVESTIGATIONAL PLAN

10.1 Overall Study Design and Plan

This is a Phase I, randomised, double-blind, placebo-controlled, parallel group, single-centre study involving 45 healthy participants. The aim is to evaluate a combination vaccine against enteric fever comprising the live attenuated Salmonella strain ZH9 (previously tested in a number of clinical trials as a typhoid vaccine) and its modified derivative, ZH9PA, in which the typhoid antigens LPS O:9 and Flagellin H:d have been substituted by the Paratyphi A antigens, LPS O:2 and Flagellin H:a. ZH9PA has not previously been tested in humans; therefore, the first two cohorts comprise a dose escalation of this component alone, followed by a final cohort in which the combination is tested at a single dose level.

There will be 3 cohorts:

Cohort 1 will consist of 9 participants, who will receive 3 doses of $1x10^9$ CFU of ZH9PA (6 participants) or placebo (3 participants).

Cohort 2 will consist of 18 participants, who will receive 3 doses of $1x10^{10}$ CFU of ZH9PA (12 participants) or placebo (6 participants).

Cohort 2 will not be initiated until all 9 participants of Cohort 1 have received 1 dose of ZH9PA or placebo, and the safety data, up to Day 7 are available. These safety data will be assessed by the safety review group, at which point the decision will be made as to whether proceeding with administration of the first dose of the higher dose level of ZH9PA ($1x10^{10}$ CFU) to Cohort 2 is appropriate.

Cohort 3 will consist of 18 participants, who will receive 3 doses of $1x10^{10}$ CFU of ZH9PA and $1x10^{10}$ CFU of ZH9 (12 participants) or placebo (6 participants).

Cohort 3 will not be initiated until a minimum of 14 participants of Cohort 2 have received 1 dose of ZH9PA or placebo, and the safety data, up to Day 7 are available. These safety data will be assessed by the safety review group, at which point the decision will be made as to whether proceeding with administration of the first dose of the combination of ZH9PA and ZH9 to Cohort 3 is appropriate. In the event that fewer than 18 participants are dosed in Cohort 2, the remaining participants to complete the required number will be dosed with Cohort 3 or as a straggler group.

Each cohort will start with a sentinel group that will consist of 2 participants, 1 will receive placebo and 1 will receive the relevant vaccine for that cohort. No further participants will be dosed until 24 hours after the second participant has received his/her dose. The rest of the cohort will be dosed provided there are no safety issues identified by the Investigator. This design allows maintenance of the “blind” until Day 70.

The clinical phase is anticipated to take place in Q4 2019 and Q2 2020. The end of the study is defined as last participant last visit.

The study will take place in the Clinical Pharmacology Unit of Simbec-Orion (Clinical Unit) under full medical and nursing supervision.

A schedule of all study assessments is provided in Table 10.7.1.

Screening (Day -28 to Day -1)

Screening assessments will be performed from Day -28 to Day -1 to ensure the eligibility of participants. Assessments will be performed as per Table 10.7.1.

Treatment Period (Day 0 to Day 84)

Participants will be admitted to the Clinical Unit on the morning of Day 0 with confirmation of eligibility and baseline assessments performed as per Table 10.7.1. Following a 90 minute (min) fast prior to dose administration, participants will receive the first dose on Day 0, and then continue to fast for a further 90 mins post dose administration; water will be allowed. Following the completion of the 4h post-dose assessments and at the discretion of the Investigator, the participants will be discharged from the Clinical Unit. Participants will return to the Clinical Unit on Day 3 for safety assessments as per Table 10.7.1. For the 7 days following the first dosing visit (Day 1 to Day 7) participants will be required to record their body temperature at home twice a day i.e. once in the morning and once in the evening (a thermometer and a diary will be provided to record body temperature). Participants will return to the Clinical Unit on Day 7 with the diary and to give a blood sample for the peripheral blood mononuclear cells (PBMC)/ALS assay; assessments will be performed as per Table 10.7.1. Participants will return to the Clinical Unit on Day 14 for safety assessments; assessments will be performed as per Table 10.7.1.

Participants will be admitted to the Clinical Unit on the morning of Day 21, with assessments performed as per Table 10.7.1. Following a 90-min fast prior to dose administration, participants will receive the second dose, and then continue to fast for a further 90 mins post dose administration; water will be allowed. Following the completion of the 4h post-dose assessments and at the discretion of the Investigator, the participants will be discharged from the Clinical Unit. Participants will return to the Clinical Unit on Day 24 for safety assessments as per Table 10.7.1. For the 7 days following the second dosing visit (Day 22 to Day 28) participants will be required to record their body temperature at home twice a day, i.e. once in the morning and once in the evening (a thermometer and a diary will be provided to record body temperature). Participants will return to the Clinical Unit on Day 28 with the diary and to give a blood sample for the PBMC/ALS assay; assessments will be performed as per Table 10.7.1.

Participants will be admitted to the Clinical Unit on the morning of Day 42, with assessments performed as per Table 10.7.1. Following a 90-min fast prior to dose administration participants will receive the third dose, and then continue to fast for a further 90 mins post dose administration; water will be allowed. Participants will return on Day 45 for safety assessments as per Table 10.7.1. For the 7 days following the third dosing visit (Day 43 to Day 49) participants will be required to record their body temperature at home twice a day, i.e. once in the morning and once in the evening (a thermometer and a diary will be provided to record body temperature). Participants will return to the Clinical Unit on Day 49 with the diary and to give a blood sample for the PBMC/ALS assay; assessments will be performed as per Table 10.7.1.

Participants will return to the Clinical Unit on Day 70 to provide a stool sample for bacteriology. If the stool sample is positive for any *Salmonella* strain at Day 70, it will be presumed to be one of the vaccine strains, and participants will be asked to return to the Clinical Unit to receive a course of antibiotic therapy. Participants who receive a course of antibiotics will be required to provide a repeat stool sample for bacteriology testing on Day 84, to confirm that shedding of the vaccine strains has ceased, as per Table 10.7.1.

Post-Study Follow-Up (Day 85 to Day 224)

Safety follow-up telephone calls will be performed on Days 154 and 224 following the first dose of study vaccine administration to check the general health of the participant and to review clinically relevant AEs. Clinically relevant adverse events are adverse events that required a visit to a healthcare professional. Assessments will be performed as per Table 10.7.1.

10.2 Dose Escalation Procedures and Stopping Criteria

10.2.1 Dose Escalation Procedures

For each cohort, a summary of all relevant safety (AEs, laboratory safety tests, ECG, vital signs, subject recorded body temperature, concomitant medications) data (up to 7 days post-first dose) will be produced on behalf of the Principal Investigator. Planned doses may be modified following a review of emerging data. Progression to the next dose level and dose selection will be based on the available safety data from all participants for Cohort 1 prior to dosing Cohort 2 and a minimum of 14 participants[1] for Cohort 2 prior to dosing Cohort 3. . Dose escalation will be dependent upon the accrual of acceptable safety data. If it is not appropriate to escalate the dose according to the proposed dose escalation schedule, then the same dose, an intermediate dose or a lower dose may be given following discussion between the Sponsor and the Principal Investigator (or deputy).

There will be a Telephone Conference at a pre-appointed time to involve the Simbec-Orion Project Manager and Principal Investigator (or deputy) and the Sponsor's representative(s), including the Sponsor's Responsible Physician. After discussion of all the data, the decision will be made whether to dose escalate and a written document (dose escalation approval form) signed by the Principal Investigator (or deputy) and Sponsor will be produced ratifying that decision. Full minutes, to be agreed by all parties, will be produced for each discussion regarding dose escalation and filed in the Investigator Site File (ISF). A copy of the signed dose escalation approval form will be provided to the Simbec-Orion Pharmacist and this will allow the IMP to be assembled for the next dose level.

Dose escalation stopping criteria are detailed in Section 10.2.2.2.

10.2.2 Stopping Criteria

10.2.2.1 General Stopping Criteria

The study will be discontinued if any unacceptable safety findings are identified. This decision will be made jointly by the Principal Investigator (or deputy) and the Sponsor. A written

[1] With the 12:6 ratio of active to placebo, if all 6 participants randomised to placebo are in the first 14 participants who are dosed, this will ensure the minimum of 8 participants will have received active vaccine.

document signed by the Principal Investigator (or deputy) and Sponsor will be produced ratifying the decision.

Individual participants may also be withdrawn for any of the reasons outlined in Section 10.5.5.

10.2.2.2 Dose Escalation Stopping Criteria

Dose escalation will be temporarily stopped pending evaluation of all available data if any of the following criteria are fulfilled:

- If one of the sentinel group or more than 20% of the participants in a cohort experience a severe systemic reaction (including anaphylactoid reactions) related to the ZH9PA/ZH9 vaccine (study agent).
- If one of the sentinel group or more than 20% of the participants in a cohort experience a persistent febrile reaction (>38.5°C for ≥12hours) related to the study agent.
- If one or more participants experience a serious adverse event (SAE) related to the study agent.
- If two or more participants in the same cohort, experience 'severe' non-serious adverse reactions (i.e. severe non-serious adverse events, independent of system-organ-class, considered at least possibly related, to the study agent).

Individual participants may also be withdrawn for any of the reasons outlined in Section 10.5.5.

10.3 Discussion of Study Design, including the Choice of Control Groups

This is a Phase I, first in human, randomised, double-blind, placebo-controlled, parallel group, study. Randomised, double-blind studies are the gold standard methodology to assess the safety, tolerability and efficacy of products and therefore, this design has been used for this study. In the case of this study, it will remain fully blinded until Day 70, when a stool sample is taken to assess if participants are shedding the bacterial strains of the vaccine in their faeces. The Investigators and the Monitor will be unblinded to the treatment allocation of participants who have a positive result for *Salmonella* and the participant will receive a course of antibiotics.

No benefit to participants is expected, as efficacy has not been evaluated for the ZH9/ZH9PA combination or for ZH9PA on its own; therefore, use of placebo as the control is considered acceptable in the healthy participants participating in the study. The use of the placebo control allows an assessment of the immune response and provides a background incidence of adverse events.

Previous work with ZH9 has shown that the double mutation does appropriately attenuate the bacteria, however it is known that the bacterial strains that make up the vaccine can be shed in the stools of participants. The vaccine strains are genetically modified organisms, and as such it is necessary to reduce environmental exposure and stool cultures are taken to ensure that participants are not shedding.

There remains a theoretical risk of the vaccine strains causing a systemic infection and so body temperatures will be taken for 7 days post each dose of vaccine. The vaccine strains are

sensitive to several antibiotics, which can be used to treat an infection in the unlikely event such an event occurs.

Three doses are to be studied, as vaccines usually require more than one dose to induce a robust immune response.

10.4 Selection of Study Population

45 participants will be required to complete the study.

The study is to be conducted in healthy participants, which will be the ultimate target population for a vaccine. While it is anticipated an immune response will be induced in participants receiving the vaccine candidate, it is not known whether the immune response is protective and; therefore, participants are not expected to derive any therapeutic benefit from taking part in the study. A healthy participant population with carefully considered inclusion / exclusion criteria will avoid the potential for interaction of ZH9PA/ZH9 with any underlying disease state or concomitant medication that it may be necessary for patients to take, while ensuring that participants are fit and well enough for participation in the study.

ZH9PA has not been previously administered to humans; therefore, its effects in humans are as yet unknown.

The following eligibility criteria are designed to select participants for whom protocol treatment and procedures are considered appropriate. All relevant medical conditions should be taken into consideration when deciding whether this protocol is suitable for a particular participant.

10.4.1 Inclusion Criteria

To be confirmed at Screening

1. Healthy male and female participants 18 to 45 years of age, inclusive.
2. Female participant of childbearing potential willing to use 2 effective methods of contraception, i.e., established method of contraception + condom, if applicable (unless of non-childbearing potential or where abstaining from sexual intercourse is in line with the preferred and usual lifestyle of the participant) from the first dose until 2 months after the last dose of IMP.
3. Female participant of non-childbearing potential. *For the purposes of this study, this is defined as the participant being amenorrhoeic for at least 12 consecutive months or at least 4 months post-surgical sterilisation (including bilateral fallopian tube ligation or bilateral oophorectomy with or without hysterectomy).*
4. Female participant of childbearing potential or non-childbearing potential with a negative pregnancy test at Screening.
5. Female participant of post-menopausal status confirmed by demonstrating at Screening that the serum level of the follicle stimulating hormone (FSH) falls within the respective pathology reference range. In the event a participant's menopausal

status has been clearly established (for example, the participant indicates she has been amenorrhoeic for 10 years, confirmed by medical history, etc), but serum FSH levels are not consistent with a postmenopausal status, determination of the participant's eligibility to be included in the study will be at the Investigator's discretion following consultation with the Sponsor.

6. Male participant willing to use an effective method of contraception or 2 effective methods of contraception, i.e., established method of contraception + condom, if applicable (unless anatomically sterile or where abstaining from sexual intercourse is in line with the preferred and usual lifestyle of the participant) from first dose until a stool sample tested for presence of the vaccine strains is negative .
7. Participant with a BMI of ≥ 19 or ≤34 kg/m^2 (BMI = body weight (kg) / [height (m)]^2).
8. No clinically significant history of liver or active gall bladder disease.
9. No clinically significant history of ongoing gastro-intestinal disease or abnormality.
10. No clinically significant history of previous allergy / sensitivity to ZH9/ZH9PA or sodium bicarbonate.
11. No clinically significant history of anaphylactic shock following vaccination.
12. No clinically significant history of hypersensitivity (e.g., hives/rash/swollen lips/difficulty with breathing) to azithromycin, ampicillin, trimethoprim-sulfamethoxazole or ciprofloxacin.
13. No clinically significant abnormal laboratory test results (in the opinion of the investigator) for serum biochemistry, haematology and/or urine analyses within 28 days before receiving the first dose administration of the IMP.
14. Participant with a negative urinary DOA screen (including alcohol and cotinine) test results, determined within 28 days before the first dose administration of the IMP unless there is a documented medical explanation for the positive result other than drugs of abuse (e.g., the participant has been prescribed opioids for pain). (N.B.: A positive test result may be repeated at the Investigator's discretion).
15. Participant with negative HIV, HBsAg and HCV Ab test results at Screening.
16. No clinically significant abnormalities in 12-lead electrocardiogram (ECG) or vital signs determined within 28 days before first dose of IMP.
17. Participant must be available to complete the study (including all follow up visits).
18. Participant must be willing to consent to have data entered into TOPS.
19. Participant must provide written informed consent to participate in the study.

To be re-confirmed on Day 0 / prior to each dosing visit

1. Participant continues to meet all screening inclusion criteria.
2. Participant with a negative urinary drugs of abuse screen (including alcohol and cotinine) prior to dosing unless there is a documented medical explanation for the

positive result other than drugs of abuse (e.g., the participant has been prescribed opioids for pain). (N.B.: A positive test result may be repeated at the Investigator's discretion).

3. Female participant of childbearing potential or non-childbearing potential with a negative pregnancy test on admission.

10.4.2 Exclusion Criteria

To be confirmed at Screening

1. Participant with any clinically significant medical (cardiovascular disease, pulmonary, hepatic, gallbladder or biliary tract, renal, haematological, gastrointestinal, endocrine, immunologic, dermatological, neurological, autoimmune disease or current infection) or psychiatric condition (see also exclusion criterion number 21) that, in the opinion of the Investigator, precludes participation in the study. This will include any clinically significant abnormal serum biochemistry results and/or haematological results and/or urine analytical results.
2. Participant with a history of heart disease or of rheumatic fever.
3. Participant with a significant acute febrile illness (including fever of 38.0°C or greater within 14 days) of each dose of IMP (Days 0, 21 and 42).
4. Participant who has chronic diseases: Chronic diseases will include all autoimmune and immunocompromising conditions and any other chronic condition, which at the judgment of the Investigator, may put the participant at higher risk of side effects from the study vaccine. Conditions in the latter category might include unexplained anaemia, hepato-biliary disease, uncontrolled hypertension, participant with prosthetic joints or heart valves, etc.
5. Participant with sickle cell anaemia.
6. Participant who has undertaken a course of antibiotics/antibacterials within 28 days prior to each dose of IMP (Days 0, 21 and 42).
7. Use of prescription or non-prescription drugs within 28 days or 5 half-lives (whichever is longer) prior to receiving the first dose of IMP, unless in the opinion of the Investigator and Sponsor's Responsible Physician the medication will not interfere with the study procedures or compromise participant safety.
8. Participant who uses antacids, proton pump inhibitors or H_2 blockers on a regular basis or has consumed proton pump inhibitors or H_2 blockers within 24 hours prior to each dose of IMP.
9. Participant who has received investigational or licensed vaccines in the 28 days prior to dosing or anticipates receiving a vaccine other than study medication up to Day 84 of the study.
10. Participant with symptoms consistent with Typhoid fever concurrent with travel to countries where typhoid infection is endemic (most of the developing world) within 2 years prior to first dose of IMP.

11. Vaccination against Typhoid within 3 years prior to first dose of IMP.
12. Ingestion of Typhoid bacteria in a challenge study within 3 years prior to dosing.
13. Participant who works as a commercial food handler.
14. Participant who is a health care worker in direct contact with patients.
15. Participant who is a childcare worker.
16. Participant who has household contact with immuno-compromised individuals, pregnant women, children < 2 years of age or individuals > 70 years of age.
17. Participant who has person(s) living with him/her who, in the opinion of the Investigator, may be at risk of disease if exposed to the vaccine strain.
18. Participant with a known impairment of immune function or receiving (or have received in the 6 months prior to study entry) cytotoxic drugs or immunosuppressive therapy (including systemic corticosteroids).
19. Participant who is current smoker (cigarettes, tobacco and/or e-cigarettes) or have stopped smoking in the last 3 months prior to Screening.
20. A clinically significant history of drug or alcohol abuse [defined as the consumption of more than 14 units of alcohol a week] within the past two years prior to Screening.
21. Inability to communicate well with the Investigators (i.e., language problem, poor mental development or impaired cerebral function).
22. Participation in a NCE clinical study within the previous 3 months or a marketed drug clinical study within the 30 days before the first dose of IMP. *(Washout period between studies is defined as the period of time elapsed between receiving the last dose of the previous study and receiving the first dose of the next study).*
23. Donation of 450 mL or more blood within the 3 months before the first dose of IMP.
24. Participant who, in the opinion of the Investigator, is unsuitable for participation in the study.

To be re-confirmed at Day 0 / prior to each dosing visit

1. Development of any exclusion criteria since the Screening visit.
2. Use of prescription or non-prescription drugs since Screening, unless in the opinion of the Investigator and Sponsor's Responsible Physician, the medication will not interfere with the study procedures or compromise participant safety.
3. Participation in a clinical study since Screening.
4. Donation of 450 mL or more blood since Screening.

10.5 Additional Advice and Restrictions for Study Population

10.5.1 Contraception

To prevent pregnancy, female participants of childbearing potential and male participants and their female partner(s) must use 2 reliable forms of contraception, i.e.,

- Condom + Established use of oral, injected or implanted hormonal contraceptive.
- Condom + Intrauterine device.
- Condom + Diaphragm with spermicide.
- True abstinence, when this is in line with the preferred and usual lifestyle of the participant. *[Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception].*

To prevent exposure of any partner (male or female) during non-vaginal intercourse to the semen from a male participant who has been exposed to the IMP, the following contraception must be used:

- Condom.

The chosen contraception method(s) must be followed from receiving the first dose of IMP until a stool sample tested for presence of the vaccine strains is negative for male participants and at least 2 months after receiving the last dose of IMP for female participants.

Male participants must not have unprotected sexual intercourse with a female who is pregnant or breastfeeding during the study.

10.5.2 Sperm Donation

Male participants must not donate sperm from receiving the first dose of IMP until a stool sample tested for presence of the vaccine strains is negative.

10.5.3 Diet and Fluid Restrictions

10.5.3.1 Meal Times/Fasts

Participants will fast for 90 mins prior to dose administration and 90 mins following dose administration. Water is permitted.

10.5.3.2 Fluid Intake

Water is allowed *ad libitum*.

10.5.3.3 Alcohol Intake

The consumption of alcohol will be limited to a maximum of 2 units per day from 7 days prior to the first dose of IMP. Alcohol will be avoided completely for a period of not less than 2 days prior to screening and each of the 3 doses of IMP. Any deviation outside this alcohol intake restriction will be assessed on a case-by-case basis at Investigator's discretion (provided the participant's alcohol intake will not impact in the safety aspects and objectives of the study and the participant has a negative alcohol screen prior to dosing).

10.5.3.4 Caffeine

Food or drink containing caffeine, including coffee, tea, cola, energy drinks or chocolates will be avoided completely whilst the participants are in attendance at the Clinical Unit (during each dosing period).

10.5.3.5 Poppy and Sesame Seeds

Participants will be advised that they must not eat food containing poppy or sesame seeds for 3 days before each visit to the Clinical Unit, as consumption of poppy seeds can lead to a positive opiate result in the drugs of abuse test.

10.5.4 Other Life-Style Restrictions

10.5.4.1 Strenuous Exercise

Strenuous exercise must be avoided completely from 3 days before receiving the first dose of IMP until completion of the Day 84 visit.

10.5.4.2 Blood Donation

Participants will be advised that they should not donate blood for at least 3 months after receiving the last dose of IMP (Day 42).

10.5.4.3 Travel Restrictions

Participants will be advised that they should not travel outside of the UK until a stool sample cultured for *Salmonella* produces a negative result to confirm that shedding of the presumed vaccine strain(s) has ceased.

10.5.5 Removal of Participants from Therapy or Assessment

Each participant will be informed of his/her right to withdraw from the study at any time and for any reason.

An Investigator will withdraw a participant from the study at any time for any of the following reasons:

- If a participant experiences a serious or intolerable AE, that prevents him/her from continuing participation in the study.
- If a participant incurs a significant protocol violation which impacts on his/her safety or the scientific integrity of the study (this will be discussed on a case-by-case basis with the Sponsor).
- At the request of the Sponsor.
- If it is considered that the participant's health is compromised by remaining in the study or the participant is not sufficiently cooperative.
- If a participant is lost to follow-up.

Participants who meet one or more of the following stopping criteria will receive no further doses of the study vaccine but will be followed up for safety:

- Severe systemic reaction, e.g., anaphylaxis.
- Persistent febrile reaction (≥38.5°C for ≥12 hours) at the Investigator's discretion
- SAE related to the study agent (serious adverse reaction (SAR) or suspected unexpected serious adverse reaction (SUSAR))

The medical safety of the participant is of paramount importance when discussing study continuation.

The reasons for any participant withdrawal will be recorded on the study completion form of the electronic case report form (eCRF).

If a participant is withdrawn or chooses to withdraw from the study for any reason every possible effort will be made to perform the evaluations described for the post-study follow-up (see Table 10.7.1). The data collected from withdrawn participants will be included in the study report.

In the event of any abnormalities considered to be clinically significant, participants will be followed up with appropriate medical management until values are considered to be clinically acceptable. Referral or collaborative care will be organised if considered necessary.

A total of 45 participants are required to complete the study. Participants who withdraw from the study before receiving any IMP will be replaced. Participants who are withdrawn from the study due to significant drug-related AEs will not be replaced. Replacement of all other participants withdrawn from the study after receiving IMP will be decided on a case-by-case basis by the Principal Investigator (or deputy) and Sponsor.

10.6 Investigational Medicinal Product

10.6.1 Identity

[redacted]

[redacted]

10.6.2 Receipt and Storage

The Sponsor must notify the Principal Investigator, or the Project Manager, prior to dispatch of IMP supplies, and of the anticipated date of their arrival. IMP should arrive at the study site at least 7 days before the first dosing day. The Sponsor shall address all supplies to:

The Production Manager

The Pharmacy
Simbec-Orion Clinical Pharmacology Unit
Merthyr Tydfil Industrial Park
Merthyr Tydfil CF48 4DR, UK

Upon receipt, supplies will be dealt with as per Simbec-Orion SOP SR-IMP 053. Temperature monitors included with shipments will be downloaded. The Sponsor will confirm that the transportation conditions are acceptable.

The IMPs will be stored securely under quarantine in a segregated, study-specific location, at < -70°C. The shipping documentation and bulk product Qualified Person (QP) certification will be reviewed. The supplies will subsequently be removed from quarantine and approved for use.

10.6.3 Assembly and Release

The IMP will be assembled into unit doses by suitably trained Simbec-Orion staff according to the Simbec-Orion SOP SR-IMP 015.

The IMP will be labelled as specified in Annex 13 (manufacture of IMPs) of the European Commission guide to Good Manufacturing Practice (GMP)[13].

[redacted]

10.6.4 Administration

10.6.4.1 Placebo Training

Each participant will be administered with 150 mL of sodium bicarbonate solution 2% weight/volume (w/v) at the Screening Visit, in order to practice oral administration prior to enrolment on the study.

10.6.4.2 Treatment Period

The following IMPs will be administered:

Cohort 1 (n=9)	3 doses of $1x10^9$ CFU of ZH9PA (6 participants) Placebo (3 participants)
Safety Review Meeting (up to Day 7 safety data)	
Cohort 2 (n=18)	3 doses of $1x10^{10}$ CFU of ZH9PA (12 participants) Placebo (6 participants)
Safety Review Meeting (up to Day 7 safety data)	
Cohort 3 (n=18)	3 doses of $1x10^{10}$ CFU of ZH9PA and $1x10^{10}$ CFU of ZH9 (12 participants) Placebo (6 participants)

The IMPs will be administered following 90 mins of fasting (water is permitted).

A dose leader design will be implemented with 2 participants being dosed on the first dosing day of EACH cohort. Of these 2 participants, 1 will be randomised to receive active drug and 1 randomised to receive placebo. The remainder of the cohort will be randomised and dosed at least 24 h later pending an acceptable safety profile in the dose-leader group and will contain at least 1 additional placebo participant. The decision to proceed will be confirmed by the Investigator or delegate and communicated with the Sponsor. This design allows maintenance of the “blind”.

IMP administration will be documented in the eCRF.

There will be 21 days between doses.

10.6.5 Return/Destruction

All used IMP containers and unused IMP (with the exception of any prepared solution that is not used for dosing) will be held under quarantine pending return/destruction.

The Sponsor must provide approval for return/destruction of all remaining IMP within 8 weeks of study completion.

All returns will be arranged at the earliest available delivery date.

For IMP destruction, the Sponsor will receive the Certificate of Destruction 4 to 6 weeks from the date of removal from site.

10.6.6 Method of Assigning Participants to Treatment Groups

Participants will be allocated to treatment groups according to a randomisation code produced by Simbec-Orion using the PROC PLAN procedure of Statistical Analysis Software (SAS®) (version 9.3 or higher). The randomisation code will include 2 dose-leaders (1 active:1 placebo) in each cohort.

Participants will be numbered sequentially from 001 (i.e. 001, 002 etc.). Replacement participants will be assigned the same randomisation as the participant they are replacing, however, 100 will be added to the number (i.e. 101 would replace 001 etc.).

10.6.7 Selection of Doses in the Study

ZH9 is a live attenuated, oral vaccine, against typhoid fever. It was previously identified as MICRO-TY. ZH9 and consists of the vaccine strain, S. Typhi (Ty2 aroC^{-} ssaV^{-}) ZH9.

[redacted]

Individual participant treatments will be dispensed by the pharmacist or designee and labelled in accordance with Annex 13 of "The Rules Governing Medical Products in European Community, volume 4 Good Manufacturing Practice for Medicinal Products"[4].

The following dosing strategy will be employed:

Cohort	Dose schedule	Dose level
1	Day 0, 21, 42	1x10^{9} CFU ZH9PA or placebo
2	Day 0, 21, 42	1x10^{10} CFU ZH9PA or placebo
3	Day 0, 21, 42	1x10^{10} CFU ZH9PA and 1x10^{10} CFU ZH9 or placebo

The dose levels chosen are based on the previous clinical experience with ZH9. These studies have determined that a dose of $1x10^{10}$ CFU is well tolerated and induces immune responses to LPS 0:9. ZH9PA is derived from ZH9, being modified to carry heterologous antigens. Other potential products, derived from ZH9, have been used to deliver heterologous antigens in clinical trials i.e. ETEC LT antigen and Hepatitis B core antigen, at doses of $1x10^{9}$ CFU and $1x10^{10}$ CFU respectively. Based on these data it is considered appropriate that the starting dose for ZH9PA is $1x10^{9}$ CFU. To date, ZH9 has been administered at a maximum dose of $1.7x10^{10}$ CFU with no significant adverse effects; therefore, administering a maximum bacterial load of $2x10^{10}$ CFU, of ZH9PA with ZH9 is considered appropriate.

10.6.8 Timing of Dose for Each Participant

Two (2) dose leaders (1 active:1 placebo) will be dosed first in each cohort. The remainder of the cohort will be dosed at least 24 h later pending an acceptable safety profile in the dose-leader group. A telephone call will be made to the dose leaders on the morning of Day 1 to assess the safety profile.

10.6.9 Blinding

A designated individual from the IMP Management Department at Simbec-Orion will generate the randomisation code under the guidance of a statistician. An independent unblinded dosing team will be assigned at the site to supervise administration of the IMP or placebo. This dosing team will have no other involvement in the study. All other site and Sponsor personnel involved in the study will be blinded with regard to the IMP being administered. The Pharmacist (or designee) responsible for the preparation of participant doses and emergency code break envelopes will not be blinded and a copy of the original randomisation code will be issued to the pharmacist (or designee) for this purpose.

Participant doses: Once the randomisation code has been authorised as per Simbec-Orion SOPs, each participant dose will be packaged and labelled for individual participants by designated individuals from the IMP Management Department at Simbec-Orion on behalf of the Sponsor.

Code break envelopes: Once the randomisation code has been authorised as per Simbec-Orion SOPs, the Pharmacist (or designee) will produce individual sealed code-break envelopes that contain the treatment allocation(s) for each participant. The envelopes will be stored in the restricted access pharmacy. A set of code break envelopes will also be provided to the person responsible for PV.

Emergency unblinding: Where the site requires emergency access to an individual participant code Simbec-Orion will break the blind via the code break envelopes stored in the pharmacy without prior consultation with the Sponsor. In such an event, the Sponsor's Responsible Physician will be notified as soon as possible via email. Please refer to Simbec-Orion SOP SR-CPU 041.

Non-emergency unblinding: If an Investigator believes that knowledge of the IMPs received by a participant is essential for appropriate treatment of an AE, the code will be broken via the code break envelopes stored in the pharmacy. Where practical, the Investigator should ideally

consult the Sponsor before breaking the code. In any event, the Sponsor will be informed as soon as practical whenever the code has been broken for a participant.

If the blind needs to be broken for an individual participant, the date and reason will be recorded in the participant's eCRF. The Investigator will not reveal the unblinded treatment code to any other member of the clinical team involved in the study or to the Study Monitor. If the code is broken for any individual participant, the participant will be withdrawn from the study and the procedures accompanying withdrawal performed. If the code is broken without justification, this will be deemed a serious protocol deviation.

10.6.10 Prior and Concomitant Therapy

Prior Medication: Prescription or non-prescription drugs, should not be taken within 28 days (or 5 half-lives (whichever is longer)) prior to receiving the first dose of IMP, unless in the opinion of the Investigator and Sponsor's Responsible Physician the medication will not interfere with the study procedures or compromise participant safety. Prescription or non-prescription drugs taken during the 28 days before receiving the first dose of IMP, and the reason for taking them, will be noted in the participant's eCRF.

Inclusion of participants who have taken prior medication will be reviewed on a case-by-case basis in relation to the safety aspects and objectives of this study.

Concomitant Medication: Prescription or non-prescription drugs should not be taken throughout the duration of the study, with the exception of paracetamol (which may be taken as an analgesic to a maximum of 2 grammes (g) in 24 h).

If intake of ANY medication is necessary during the study, the daily dosage, duration and reasons for administration will be recorded in the concomitant medication section of the participant's eCRF.

Prohibited Medications:

28 days prior to first dose (Day 0) to Day 49:

- Prescription and Non-prescription drugs (exception of 2 g per 24 hours of paracetamol).

28 days prior to first dose (Day 0) to 28 days following the third dose on Day 42, i.e. Day 70:

- Antibiotics

  Participants requiring antibiotics to treat an infection from Day 0 to Day 49 will be followed for safety but will not receive further doses of IMP.

28 days prior to first dose (Day 0) to Day 84:

- Non-steroidal anti-inflammatory drugs (NSAIDs) (participants will be advised to avoid NSAIDs if possible)
- Steroids
- Immunosuppressants

- Vaccines

10.6.11 Treatment Compliance

Each dose of IMP will be taken under supervision and compliance will be documented. The exact time a participant starts to drink the IMP and the exact time a participant finishes drinking the IMP will be recorded on the participant's eCRF.

10.7 Safety Variables

10.7.1 Safety Measurements Assessed and Flow Chart

A schedule of study assessments is provided in Table 10.7.1.

Simbec-Orion personnel who have been appropriately trained will carry out study procedures.

Where more than 1 procedure is scheduled for the same time-point, the following order of priority will apply:

1. Safety and pharmacodynamic (PD) blood sampling
2. Vital signs and 12-lead ECG (a window of ± 10 min in relation to the nominal time-point is allowed).
3. Urine collection for urinalysis, urine DOA and urine pregnancy (if applicable)

All baseline assessments will be performed prior to dosing on each dosing day.

Table 10.7.1 Study Flow Chart

	Screening	Treatment Period[1]												Post-Study Follow-Up Telephone Call[2]	
Week		**0**		**1**	**2**	**3**		**4**	**6**		**7**	**10**	**12**	**22**	**32**
Day	**-28 to -1**	**0**	**3**	**7**	**14**	**21**	**24**	**28**	**42**	**45**	**49**	**70**	**84**	**154**	**224**
Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Visit Window (days)	**N/A**	**+/-0**	**+/-1**	**+/-1**	**+/-1**	**+/-0**	**+/-1**	**+/-1**	**+/-0**	**+/-1**	**+/-1**	**+/-1**	**+/-7**	**+/-7**	**+/-7**
Informed Consent	X														
Inclusion/Exclusion[3]	X	X				X			X						
Demographics	X														
Height / Weight / BMI	X												X(weight only)		
Medical History & Concurrent Conditions	X														
Virology Tests	X														
Urine DOA (including Alcohol and Cotinine)[3]	X	X[11]				X[11]			X[11]						
Pregnancy[3, 4]	X(serum)	X(urine) 11				X(urine) 11			X(urine) 11				X(serum)		
FSH[5]	X														
Biochemistry[3]	X	X[11]		X		X[11]		X	X[11]		X		X		
Haematology[3]	X	X[11]		X		X[11]		X	X[11]		X		X		
Urinalysis	X		X				X			X			X		
Vital Signs[6]	X	X	X	X	X	X	X	X	X	X	X		X		
Physical Examination	X												X		
General Health Check		X				X			X						
12-Lead ECG	X												X		
Placebo training	X														
Vaccine administration[7]		X				X			X						

	Screening	Treatment Period[1]												Post-Study Follow-Up Telephone Call[2]	
Week		**0**		**1**	**2**	**3**		**4**	**6**		**7**	**10**	**12**	**22**	**32**
Day	**-28 to -1**	**0**	**3**	**7**	**14**	**21**	**24**	**28**	**42**	**45**	**49**	**70**	**84**	**154**	**224**
Visit	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
Visit Window (days)	**N/A**	**+/-0**	**+/-1**	**+/-1**	**+/-1**	**+/-0**	**+/-1**	**+/-1**	**+/-0**	**+/-1**	**+/-1**	**+/-1**	**+/-7**	**+/-7**	**+/-7**
Twice-Daily Temperature Records[8]		←	———	→		←	———	→	←	———	→				
Blood culture[9]		←	———	———	———	———	———	———	———	———	———	———	———	→	
Stool bacteriology (vaccine strains)[10]												**X**	**(X)**		
Administration of antibiotics[10]												**(X)**			
Blood sample for serum Ig responses[11]		**X**				**X**			**X**				**X**		
PBMC collection for ALS assay[11]		**X**		**X**				**X**			**X**				
Adverse Events		**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**
Concomitant Medication	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**

Study Flow Chart Footnotes:

All procedures on dosing days (Days 0, 21, 42) are to be completed prior to dosing (except post dosing vital signs and health check for discharge)

[1] The treatment period will be approximately 85 days duration, from the first dosing day (Day 0) until Day 84 (inclusive).

[2] Post-study follow-up telephone call will be performed on Days 154 and 224 after receiving the first dose of IMP (Day 0).

[3] Eligibility will be confirmed prior to dose administration on Day 0, Day 21 and Day 42.

[4] For female participants only.

[5] For postmenopausal female participants only

[6] At Day 0, Day 21, and Day 42, vital signs to be performed pre-dose and at 4 hours post-dose.

[7] IMP will be administered fasted (90 mins prior to dose administration). Participants will fast for a further 90 mins post each dose administration. Participants in sentinel cohorts will be contacted by telephone approximately 24 hours following the Day 0 dose to assess their safety profile.

[8] Participants to record body temperature twice daily at home for 7 days post administration of each dose of IMP.

[9] Blood cultures to be performed to identify vaccine strains only if a participant experiences defined symptoms (e.g., fever greater than 37.8°C, white blood cell (WBC) $>20x10^9$ cells/L, respiratory rate >25 breaths per min, pulse rate >130 beats per min (bpm)) or clinically indicated.

[10] Participants will attend the Clinical Unit on Day 70 to provide a stool sample. If the stool sample is positive for any *Salmonella* strain at Day 70, it will be presumed to be one of the vaccine strains, and participants will be asked to return to the Clinical Unit to receive a course of antibiotic therapy, and a repeat stool culture will be obtained on Day 84 to confirm shedding of *Salmonella,* presumed to be one of the vaccine strains, has ceased.

[11] Samples to be collected prior to each dosing.

10.7.1.1 Demographic and Background Assessments

The following demographic and background assessments will be performed during the study at the time-points specified in Table 10.7.1.

10.7.1.1.1 Demographics

Demographic data: age, year of birth, gender, race, height, weight and BMI.

Height in metres (m) (to the nearest centimetre (cm)) and weight in kilogram (kg) (to the nearest 0.1 kg) in indoor clothing and without shoes will be measured. BMI = body weight (kg) / [height (m)]^2 will be calculated.

10.7.1.1.2 Medical History and Concurrent Conditions

Relevant medical history and current conditions will be recorded in the eCRF.

10.7.1.1.3 Virology Tests

Virology tests: HBsAg, HCV Ab and HIV (antibodies to HIV-1 and HIV-2).

HBsAg, HCV Ab, and HIV (antibodies to HIV-1 and HIV-2) virology tests will be analysed from the same serum sample for biochemistry analyses at Screening by Simbec-Orion Laboratory Services , using the Roche cobas® c6000 analyser series comprising of the c501 and e601 modules or any other appropriate analyser.

10.7.1.1.4 Drugs of Abuse (including Alcohol and Cotinine)

Urine DOA screen (including alcohol and cotinine): Alcohol, amphetamines, barbiturate, benzodiazepine, cannabinoids, cocaine, cotinine, methadone, and opiate.

A **mid-stream** urine sample will be collected into a universal collection/storage container. At protocol-defined time-points when both urinalysis and drugs of abuse / alcohol screening are required, all urine analyses will be performed from a single approximately 20 mL urine sample.

Urine samples for drugs of abuse (including alcohol and cotinine) will be analysed by Simbec-Orion Laboratory Services, using the Roche cobas® c6000 analyser series comprising of the c501 and e601 modules or any other appropriate analyser. Assessments of urine sample quality (i.e., urine sample verification) will be performed by measuring urine creatinine for DOA.

10.7.1.1.5 Pregnancy Test, Menstrual and Obstetric History

Pregnancy tests will be performed on all female participants. Pregnancy tests will be performed by Simbec-Orion Laboratory Services. Serum pregnancy tests will be performed at time-points specified in Table 10.7.1. Urine pregnancy tests will be performed manually using appropriate urine pregnancy test kits at time-points specified in Table 10.7.1.

Serum FSH to confirm post-menopausal status will be analysed from the same serum sample for biochemistry analyses at Screening for postmenopausal females only**.** Serum FSH analysis will be performed by Simbec-Orion Laboratory Services using the Roche cobas® c6000 analyser series comprising of the c501 and e601 modules or any other appropriate analyser.

10.7.1.2 Compliance with Inclusion/Exclusion Criteria

An Investigator will assess all participants against the study inclusion and exclusion criteria at Screening. Compliance will be re-confirmed on prior to each dose administration.

10.7.1.3 Safety Assessments

The following safety assessments will be performed at the time-points specified in Table 10.7.1.

10.7.1.3.1 Adverse Events

AEs and SAEs that occurred during the study along with their severity and relationship to study drug will be reported.

An AE is defined as per Statutory Instrument 2004 No. 1031[4]:

Any untoward medical occurrence[1] in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.[2]

Notes:

[1] Whether subjective complaint or objective finding.

[2] An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporarily associated with the use of an IMP, whether or not considered related to the IMP.

An unexpected adverse reaction is defined as:

An adverse reaction, the nature, or severity of which is not consistent with the applicable product information (e.g., Investigator's brochure for an unapproved investigational product of summary of product characteristics (SmPC) for an authorised product).

AEs will be monitored throughout the study from the signature of participant informed consent through to the post-study follow-up visit. All AEs will be recorded, whether considered minor or serious, drug-related or not.

All abnormal laboratory findings considered to be clinically significant will be recorded as AEs.

Recording of Adverse Events

All of the following details will be recorded in the participant's eCRF for each AE:

- Full description of AE.
- Date and time of onset.
- Date and time of resolution.
- Severity of event to be assessed by an Investigator in accordance with the definitions below.
- Relationship to IMP to be assessed by an Investigator in accordance with the definitions below.
- Action taken (if any).
- Outcome and details of any further follow-up.

Grades of Adverse Event Severity

The following grades will be used by an Investigator to describe the severity of AEs.

The following are the only grades, which will be used to describe AE severity. Only 1 severity grade will be used for each AE (e.g., mild - moderate is not acceptable).

SEVERITY OF THE AE	DEFINITION
Mild	The AE does not interfere with the participant's daily routines. It causes no more than slight discomfort or mild objective change in any of the safety parameters assessed during the study as determined by the Investigator.
Moderate	The AE interferes with some aspects of the participant's daily routines or moderate objective change in any of the safety parameters assessed during the study as determined by the Investigator.
Severe	The AE causes inability to carry out the participant's daily routine or severe objective change in any of the safety parameters assessed during the study as determined by the Investigator.

Definitions of Adverse Event Causality

The following definitions will be used by an Investigator to describe the relationship between an AE and the IMP.

The following are the only definitions which will be used to describe the relationship between AEs and the IMP. Only 1 relationship definition will be used for each AE (e.g., possible-probable is not acceptable).

RELATIONSHIP TO IMP	DEFINITION
Reasonable possibility of being related	• There is a reasonable possibility of the event being related to the IMP. This might be temporal or due to a physiological or pharmacodynamic process
No reasonable possibility of being related.	• No reasonable possibility of the event being related to the IMP, the IMP may not have been administered, no temporal relationship, and no known or understood physiological or pharmacological mechanism for the event to be related.

Adverse events of special interest

For this study, adverse events of special interest are:

- Bacteraemia proven to be caused by one of the vaccine strains
- Development of autoimmune diseases.

Should one of these events occur the PI (or delegate) will alert the Sponsor's Responsible Physician within 24 hours of becoming aware.

Serious Adverse Events

SAEs are defined in Statutory Instrument 2004 No. 1031 as any AE that

a. results in death,

Note: Death is an outcome (of an AE, of progressive disease, etc.) and not an AE in itself.

b. is life-threatening,

A life-threatening event places the patient at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe.

c. requires hospitalisation or prolongation of existing inpatients' hospitalisation,

"In-patient hospitalisation" means that the patient has been formally admitted to a hospital for medical reasons, for any length of time. It does not include presentation to or care within an emergency department.

Complications that occur during hospitalisations are AEs. If a complication prolongs hospitalization, it is an SAE.

d. results in persistent or significant disability or incapacity, or

e. consists of a congenital anomaly or birth defect.

f. Is a medically important event or reaction.

Some medical events may jeopardise the participant or may require an intervention to prevent one of the above characteristics/consequences. Such events (referred to as 'important medical events') should also be considered as 'serious' in accordance with the definition.

SAEs must be reported within 24 h of knowledge of the event by submitting an initial SAE report via email or fax to Diamond Pharma Services Ltd.

E mail: pvservices@diamondpharmaservices.com

SAE Fax: +44 (0) 1279 418964

Suspected Unexpected Serious Adverse Reactions

AEs which meet all of the following criteria

- Serious.
- Unexpected (i.e. is not consistent with the applicable product information e.g., Investigator's brochure for an unapproved IMP or SmPC) for an authorised product.
- There is at least a reasonable possibility that there is a causal relationship between the event and the medicinal product (possible, probable, almost definite).

will be classified as SUSARs and will be reported to the REC and to the MHRA in accordance with applicable regulatory requirements for expedited reporting. It is the Sponsor's responsibility to report SUSARs to the REC and MHRA.

To ensure no confusion or misunderstanding of the difference between the terms "serious" and "severe," which are not synonymous, the following note of clarification is provided:

The term "severe" is often used to describe the severity of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). This is not the same as "serious," which is based on participant/event outcome or action criteria usually associated with events that pose

a threat to a participant's life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

Monitoring of Participants with Adverse Events

In the event of any abnormalities considered to be clinically significant by the investigating physician, participants will be followed up with appropriate medical management until:

- It has resolved/returned to normal or baseline.
- The event has stabilised at a level acceptable to the Investigator and is not considered to be clinically significant.

Pregnancy

Pregnancies must be reported within 24 h of knowledge and should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or AEs that occur in the new born child(e.g., meconium ileus, respiratory distress).

Pregnancy outcomes must be collected for females who took the IMP and the female partners of any males who took the IMP. Consent to report information regarding these pregnancy outcomes should be obtained from the mother.

10.7.1.3.2 Laboratory Safety Assessments

Laboratory Safety Screen

Laboratory safety screen samples will be analysed by Simbec-Orion Laboratory Services. Printed laboratory test result reports will include normal reference ranges. A decision regarding whether the laboratory test result outside the normal reference range is of clinical significance or not shall be made by an Investigator/designee and the report will be annotated accordingly. Clinically significant laboratory test result abnormalities will be recorded on the AE page. The normal reference ranges for laboratory test parameters will also be filed in the ISF.

Biochemistry Tests: Alanine transaminase, albumin, alkaline phosphatase, aspartate transaminase, bicarbonate, total bilirubin, direct bilirubin, calcium, cholesterol, chloride, creatinine, creatine kinase, c-reactive protein, glucose (random), gamma glutamyl transferase, total globulin, lactate dehydrogenase, potassium, inorganic phosphorus (or phosphate), total protein, triglycerides, sodium, urea and uric acid.

Blood samples for biochemistry analyses for each time-point will be collected into an appropriately sized serum collection tube with or without a separator, and analysed by Simbec-Orion Laboratory Services using the Roche cobas® c6000 analyser series comprising of c501 and e601 modules or any other appropriate analyser. Assessments of blood sample quality (i.e., for sample verification) will be performed by measuring 3 indices [namely, Lipaemic (for Lipaemia), Haemolytic (for Haemolysis) and Icteric (for Icterus)] in serum.

Haematology Tests: Haemoglobin, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, platelets, red blood cell count, red blood cell distribution width, WBC count, and WBC differential count (neutrophils,

lymphocytes, monocytes, eosinophils, and basophils) (reported in absolute and percentage values).

Blood samples for haematology analyses for each time-point will be collected into a collection tube containing ethylenediaminetetraacetic acid and analysed by Simbec-Orion Laboratory Services using the Siemens ADVIA® 2120 or Siemens ADVIA® or an any other appropriate analyser.

Urinalysis Tests: Specific gravity, pH, protein, glucose, ketones (or ketone bodies), urobilinogen, bilirubin, blood, leukocytes, and nitrite.

A mid-stream urine sample for each time-point will be collected into a universal collection/storage container and divided into aliquots. Urinalysis will be performed by Simbec-Orion Laboratory Services, using the Roche cobas® U6500 or any other appropriate analyser.

In the event that the urinalysis automated 'dipstick' test result is positive for nitrites and/or 2+ or more reported for protein, blood, and/or leucocytes, then urine microscopy will be performed. The following test parameters will be reported: bacteria, casts (non-pathogenic), casts (pathogenic), crystals, epithelial cells, red blood cells and white blood cells. The urine microscopy will be performed by Simbec-Orion Laboratory Services, using the Roche cobas® U6500 701 module or any other appropriate analyser/method. Separate aliquot samples will be sent for culture, if microscopy visualises bacteria (please refer to the Sample Handling Manual for processing and shipping instructions).

10.7.1.3.3 Other Safety Assessments

Vital signs: Systolic/diastolic blood pressure, pulse rate, oral body temperature. Measurements will be recorded in the supine position. Blood pressure, pulse rate and oral body temperature will be measured by the DINAMAP* Compact Vital Signs Monitor (Model TS) or equivalent. Normal ranges for vital signs are presented in Appendix 2.

Physical Examination: A physical examination will be performed by an Investigator. The examination will include ear/nose/throat, ophthalmological, dermatological, cardiovascular, respiratory, gastrointestinal, central nervous system, lymph nodes and musculoskeletal). An Investigator can examine other body systems if required, at their discretion.

12-Lead ECG: Heart rate, PR interval, QRS width, QT interval and QT interval corrected using Bazett's formula and Fridericia's formula.

12-lead ECG recordings will be made using a MAC 5500 or equivalent. Each ECG trace will be labelled with the study number, participant number, participant year of birth. An Investigator will provide an interpretation of each tracing. Clinically significant abnormalities will be recorded on the AE page. Normal ranges for 12-lead ECG parameters are presented in Appendix 2.

10.7.1.3.4 Stool Bacteriology

Stool samples of up to 10 g of stool will be collected for stool bacteriology (vaccine strains) in appropriate stool collection kits at the timepoints specified in Table 10.7.1.

Detailed sample collection and processing instructions will be documented in the SHM.

10.7.1.3.5 Blood Cultures

Blood samples will be collected (as specified in Table 10.7.1) for blood cultures to be performed to identify ZH9 and/or ZH9PA only if the participant experiences defined symptoms (e.g., fever greater than 37.8°C, WBC >20 x 10^9 cells/L, respiratory rate >25 breaths per min, pulse rate >130 bpm) or clinically indicated. 8-10 ml of blood will be collection into bottles enriched with Aro. Detailed sample collection and processing instructions will be documented in the SHM.

10.7.1.3.6 Concomitant Medication

All prior and concomitant medications taken during the study will be recorded in the participant's eCRF (see Section 10.6.10).

10.7.2 Appropriateness of Measurements

All measurements performed in the study are standard measurements.

The total volume of blood to be collected from each participant during the study (approximately 388 mL) is considered acceptable (Table 10.7.2). If repeat or additional samples are required, the volume of blood taken will not exceed 500 mL.

Table 10.7.2 Summary of Blood Volume

Procedure	Visit	No. of Samples	Blood Volume per Sample (mL)
Biochemistry	Screening[1]	1	3.5
	Day 0	1	3.5
	Day 7	1	3.5
	Day 21	1	3.5
	Day 28	1	3.5
	Day 42	1	3.5
	Day 49	1	3.5
	Day 84[2]	1	3.5
Haematology	Screening	1	3.0
	Day 0	1	3.0
	Day 7	1	3.0
	Day 21	1	3.0
	Day 28	1	3.0
	Day 42	1	3.0
	Day 49	1	3.0
	Day 84	1	3.0
PD Assessment – PBMC/ALS assay	Day 0	1	64
	Day 7	1	64
	Day 28	1	64
	Day 49	1	64
PD Assessment – Serum Ig responses	Day 0	1	20
	Day 21	1	20
	Day 42	1	20
	Day 84	1	20
		Total Blood Volume[3]	388

[1] From the biochemistry blood sample collected at the Screening visit, the serum pregnancy test (for all female participants only), serum FSH (for postmenopausal female participants only) and virology screen (HBsAg, HCV Ab, and HIV) will be analysed from the same serum sample.

[2] From the biochemistry blood sample collected at Day 84, the serum pregnancy test (for all female participants only) will be analysed from the same serum sample.
[3] The exact volumes of each sample may change but the total volume of blood drawn for any participant will not exceed 500 mL. This total blood volume does not include blood samples required to be collected for performing blood cultures as specified in Table 10.7.1, and any repeat/unscheduled/additional blood sample collections required at the discretion of the Investigator/deputy.

10.7.3 Pharmacodynamic Assessments

The following PD blood samples will be collected at the time-points specified in Table 10.7.1.

- Serum Ig Responses
- PBMC for ALS assay

Detailed sample collection and processing instructions will be documented in the SHM.

The IgG and IgA responses to four antigens (LPS O:9, LPS O:2; H:d, H:a) will be determined by a validated enzyme-linked immunosorbent assay (ELISA) end point titre method.

PBMC will be cultured for 72 hours (+/- 3 hours) and the concentration of IgA in the supernatant specific for the same four antigens will be determined by a validated ELISA end point titre method.

10.7.4 Other Assessments

Not applicable.

10.8 Data Quality Assurance

At the time the study is initiated, a representative of the Sponsor will review the final protocol and eCRFs with the Principal Investigator and site staff. During the course of the study the Monitor will visit the Clinical Unit to check the completeness of the participants records (including the volunteer (participant) master files (VMF), laboratory and 12-lead ECG print-outs), the accuracy of entries into the eCRFs, the adherence to the final protocol and to ICH GCP E6 (R2) guidelines [3], the progress of enrolment and also to ensure the storage, handling and accountability of the IMP. The Principal Investigator and key study personnel will be available to assist the Monitor during these visits.

The Principal Investigator will give the Monitor, Auditor(s), the REC, and the MHRA direct access to relevant clinical records to confirm their consistency with the eCRF entries. No information in these records about the identity of the participants will leave Simbec-Orion. The Sponsor will maintain the confidentiality of all participant records, in line with Section 6.10 of the ICH GCP E6 (R2) guidelines[3] and the General Data Protection Regulation (GDPR) European Union (EU) 2016_679[14].

Study data will be fully documented in the eCRFs, study log books and paper source files. Signatures (dated via audit trail) will be given to account for all interventions in the study by research staff.

Source data is all information in original records and certified copies of original records of clinical findings, observations, or other activities in a clinical study necessary for the

reconstruction and evaluation of the study. Source data are contained in source documents (original records or certified copies).

For the purposes of this study the source data will be recorded as detailed in Table 10.8.1.

Table 10.8.1 Summary of Source Documentation Location

Data	Source Document			
	VMF	eCRF[4]	Paper Source File	Log Book
Evidence of healthy participant status for entry into clinical study	X			
Demographic data[1]	X	X		
Medical history	X			
Inclusion and exclusion criteria		X		
Informed consents[2]	X	X		
Participant participation in the clinical study	X	X		
Participant number in the clinical study		X	X	
Adverse events		X		
Previous and on-going therapy	X			
Concomitant therapy		X		
Results of study examinations (e.g., 12-lead ECGs, vital signs and laboratory safety tests)[3]		X	X	
Study visit dates		X		
Administration of IMP		X	X	
Serum PD sample collection times		X		
Serum PD sample tracking				X
Evidence of handwashing training				X
Sentinel dosing 24-hour telephone call				X

1. Only year of birth will be captured in the eCRF
2. Copies of the informed consent form should be present in the VMF. The original informed consent forms will be maintained in the study officer file during the clinical phase and will then be transferred to the Project Manager for archiving with the Investigator site file at the end of the study.
3. The 12-lead ECG trace and laboratory safety test print-out will be stored in the paper source file.
4. In the event of eCRF technical issues source data will be located in the paper source file.

The above table indicates where source data will be recorded but for completeness the following information will also be recorded in the VMF:

- Clinical study code.
- Study visit dates (pre-dose; post-dose).
- IMP administration (date of last dose).
- Results of any key safety and efficacy measures from the clinical study that, in the opinion of an Investigator, should be noted.
- Any concomitant medications used to treat the participant during the study that should be noted.

The data collected in the eCRFs during the study will be subject to quality control checking by clinical staff prior to sign off.

Designated investigator staff will enter the data required by the protocol into the eCRFs using fully validated software that conforms to 21 CFR Part 11 requirements. Staff will not be given access to the eCRF until they have been trained. Automatic validation programs check for data discrepancies and, by generating appropriate error messages, allow the data to be confirmed or corrected before transfer of the data to the Biometrics group. The Investigator must certify that the data entered into the eCRF are complete and accurate.

The study will be subject to an independent audit by the Simbec-Orion Quality Assurance Unit as outlined in Simbec-Orion SOP BD/324/9/01.

Independent clinical quality assurance audits may be performed at any time during or following completion of the study by the Sponsor, or its authorised agents, and Regulatory Authorities and/or the REC.

10.9 Statistical Methods and Determination of Sample Size

10.9.1 Statistical and Analytical Plan

A statistical analysis plan (SAP) will be written by Simbec-Orion and agreed by Prokarium prior to the locking of the database and subsequent reporting of the study data.

10.9.1.1 Study Variables/Endpoints

Primary Endpoint

- Local and systemic reactogenicity up to Day 84 as assessed by adverse events, laboratory safety tests (biochemistry, haematology, urinalysis), vital signs and physical examination.

Secondary Endpoints

- Local and systemic reactogenicity; from Day 85 to Day 224 as assessed by adverse events, laboratory safety tests (biochemistry, haematology, urinalysis) if undertaken for cause; vital signs if undertaken for cause; physical exam if undertaken for cause.
- Concentrations of specific serum IgA and IgG antibodies to LPS O:2 and O:9, flagellin H:a and H:d.
- Fold increase in specific serum IgA and IgG antibody concentrations, in individual participants, against the antigens LPS O:2 and O:9, flagellin H:a and H:d.
- Concentrations of specific mucosal IgA antibodies in lymphocyte supernatant (ALS) assays, in individual participants, against antigens LPS O:2 and O:9, flagellin H:a and H:d.
- Seroconversion rate (proportion of participants with 4-fold increase above baseline at any time post vaccination) against each of the above antigens.
- Fold increase in specific mucosal IgA antibodies in lymphocyte supernatant (ALS) assays, in individual participants, against the antigens LPS O:2 and O:9, flagellin H:a and H:d.

10.9.1.2 Analysis Sets

Safety Set: All randomised participants who receive at least 1 dose of IMP will be included in the Safety Set.

PD Set: All randomised participants who receive at least 1 dose of IMP and have at least one post-baseline antibody concentration will be included in the PD Set.

Per Protocol Set: The Per Protocol Set will be comprised of all participants in the PD Set who comply with the following criteria:

- Do not have an occurrence of vomiting or diarrhoea which may impact the systemic exposure of IMP;
- Do not use a concomitant medication which may impact the immunogenicity results;
- Do not incur a major protocol deviation which may invalidate or bias the immunogenicity results.

10.9.1.3 Description of Statistical Methods

All statistical analysis will be performed using SAS® (version 9.3 or higher).

Descriptive statistics for quantitative parameters will be provided using number of observations (N), mean, standard deviation (SD), minimum, median and maximum. Descriptive statistics for qualitative parameters will be provided using absolute frequencies (n) and relative frequencies (%). For immunological endpoints (IgG, IgA, concentrations and fold increase) additionally geometric means with corresponding 95% confidence intervals (CI) will be presented.

For parameters with evaluation before vaccination and in case of repeated value(s), only the last observation prior to dosing will be used in descriptive and inferential statistics and derivations of other parameter values. After vaccination, only values of scheduled assessments (planned in the protocol) will be used.

10.9.1.3.1 Demographic and Background Data

All demographic and background data will be listed, in addition:

Disposition: Participant disposition will be listed with any withdrawals flagged. Frequencies (number and %) of the total number of participants dosed, completed and prematurely discontinued (including reason for discontinuation) from the study will be summarised by treatment and overall (all treatments combined). Additionally, the number and percentage of participants included within each analysis set will be summarised.

Demographics: Demographic data will be listed. Descriptive statistics (number of participants in the analysis set (N), number of participants with non-missing observations (n), mean, standard deviation (SD), minimum, median and maximum) will be tabulated by treatment and overall for the continuous variables age, height, weight and BMI and frequencies (number and %) for the categorical variables race and gender.

10.9.1.3.2 Efficacy Data

Not applicable.

10.9.1.3.3 Safety Data

All safety data will be listed, in addition:

AEs: All AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA) dictionary (the most up to date version will be used and this will be documented in the DMP)

All AEs, including those which occurred prior to receiving the first dose of IMP, will be listed. Only treatment emergent adverse events (TEAEs), i.e., existing conditions that worsen or events that occur during the course of the study after administration of IMP, will be included within the summary tables. AE summaries will be presented by treatment, all active treatments combined and overall.

An overall summary of AEs will be produced including the number of TEAEs; the number and % of participants reporting at least 1 TEAE, serious TEAE, TEAE leading to withdrawal from the study; the number and % of participants reporting TEAEs by severity and relationship to IMP.

The number of TEAEs and the number and % of participants reporting at least 1 TEAE will be tabulated by system organ class (SOC) and preferred term. A participant reporting multiple episodes of a particular AE will only contribute 1 count towards the corresponding SOC and preferred term.

The number of TEAEs and the number and % of participants reporting at least 1 TEAE will be tabulated by preferred term and sorted by descending frequency on the total number of participants with that AE. A participant reporting multiple episodes of a particular AE within a treatment period will only contribute 1 count towards the corresponding preferred term.

In addition, the number and % of participants reporting TEAEs will be tabulated by maximum severity and strongest relationship to IMP. For the summary of TEAEs by severity, if a participant has multiple events occurring within the same SOC or preferred term the event with the highest severity will be counted. Similarly, for TEAEs by relationship to IMP, if a participant has multiple events occurring within the same SOC or preferred term, the event with the highest association to IMP will be counted.

The incidence of TEAEs will be compared between treatments using Fisher's Exact Test (or an alternative statistical test if appropriate).

Laboratory Safety: Biochemistry, haematology, and urinalysis parameters will be listed with any out of normal range values flagged. Laboratory test results which are out of normal range will also be presented separately along with normal reference ranges. Descriptive statistics (N, n, mean, SD, minimum, median and maximum) of absolute and change from baseline (Day 0) values at each protocol-defined time-point will be tabulated by treatment.

Vital Signs: Vital signs parameters will be listed with any out of normal range values flagged. Descriptive statistics (N, n, mean, SD, minimum, median and maximum) of absolute and change from baseline (Day 0 pre-dose) values at each time-point will be tabulated by treatment.

12-Lead ECG: 12-Lead ECG parameters will be listed with any out of normal range values flagged.

10.9.1.3.4 PD Data

Descriptive statistics (N, n, arithmetic mean, standard deviation (SD), minimum, median, inter-quartile range, maximum, geometric mean with corresponding 95% CI) of serum IgG and IgA, mucosal IgA concentrations and fold-increase will be summarised by visit for each treatment group.

The immune response (log-transformed serum IgG and IgA, mucosal IgA concentrations) will be subjected to an ANOVA/ANCOVA to compare pre-vaccination and post-vaccination levels within a treatment group for each visit. Similarly, the immune response (log-transformed serum IgG and IgA, mucosal IgA concentrations) and the magnitude of the immune response (fold-increase) will be compared between treatment groups at each visit.

In addition, the number of participants seroconverting per visit (i.e. with 4-fold increased level above baseline) will be compared between treatment groups using Fisher's Exact Test (or an alternative if appropriate).

10.9.1.3.5 Other Data

Not applicable.

10.9.2 Sample Size Calculation

The sample size chosen for this study is not based on a formal statistical estimation but is considered to be adequate to meet the objectives of the study. A sufficient number of participants will be initially screened for enrolment to ensure that the planned sample size is achieved.

11 PRACTICAL CONSIDERATIONS

11.1 Storage of Data

The Investigator site file and associated study documentation will be archived for at least 25 years after the end of the study (last participant last visit) as per European Medicine Agency Guideline INS/GCP/856758/2018[15]. The study documentation may be transferred to an offsite storage facility during this period but will remain under the control of Simbec-Orion.

The Sponsor has delegated the set up and maintenance of the Sponsor trial master file (TMF) to Simbec-Orion. The TMF will be returned to the Sponsor at the end of the study, who will archive it for at least 25 years after the end of the study.

11.2 Protocol Amendments

Changes in the study protocol must take the form of written protocol amendments and shall require the approval of all persons responsible for the study (see Section 1).

A protocol amendment is deemed to constitute a substantial protocol amendment if it is considered to be likely to affect to a significant degree either:

a. The safety or physical or mental integrity of the participants of the study.

b. The scientific value of the study.

c. The conduct or management of the study.

d. The quality or safety of any IMP used in the study.

Such amendments must be submitted to the REC responsible for the study and the MHRA for approval prior to implementation.

Protocol amendments required for urgent safety reasons may be implemented immediately. However, the REC and MHRA must be notified in writing within 3 days of the measures taken and the reasons for implementation.

All other amendments shall be deemed to be non-substantial and as such do not need the prior approval of the REC and the MHRA.

11.3 Confidentiality

The confidentiality of the study must be maintained at all times and the Principal Investigator must not reveal any information relating to the study without express permission from the study Sponsor.

11.4 Study Report and Publication Policy

Simbec-Orion will investigate and analyse the data generated with all due speed.

A draft study report will be sent to the Sponsor for review. The Sponsor will forward any comments on the draft study report to the Project Manager within 30 days of receipt. Upon receipt of these comments a final, Quality Assurance (QA) approved report will be issued with all due speed. A copy of the report will be forwarded to the Sponsor.

The Principal Investigator will obtain the Sponsor's written permission before any information concerning this study is submitted for publication.

11.5 General Data Protection Regulation

Personal data of the participant shall be processed in a manner that ensures it has appropriate security. This includes protection against unauthorised or unlawful processing and against accidental loss, destruction or damage and by using appropriate technical or organisational measures. One such measure is by the Investigator ensuring that the participants' personally identifiable information should be replaced through the use of pseudonymisation.

On the eCRFs or other documents submitted to Prokarium will NOT be identified by their names but by the assigned participant number (panel/screening/participant number) to ensure confidentiality of the participant's information and that data minimisation principles are maintained. If participant names are included in error on copies of documents submitted to Prokarium, the names will be erased or securely destroyed and the assigned participant number added to the document.

Documents not intended for submission to Prokarium/Simbec-Orion (e.g., signed consent forms) should be maintained by the Investigator in strict confidence and not disclosed to any parties outside of this approved agreement. Forms should be protected by use of strong encryption.

12 REFERENCES

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[4] The Medicines for Human Use (Clinical Trials) Regulations 2004 (Statutory Instrument 2004 No. 1031) and subsequent amendments.

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[13] European Commission. EudraLex The rules governing medicinal products in the European Union – Volume 4: Good Manufacturing Practice Guidelines.

[14] The General Data Protection Regulation (EU) 2016/679 (25 May 2018).

[15] European Medicines Agency Guideline on the content, management and archiving of the clinical trial master file (paper and/or electronic) EMA/INS/GCP/856758/2018 (06 Dec2018).

APPENDIX 1: DECLARATION OF HELSINKI (BRAZIL, 2013)

https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for medical-research-involving-human-participants/

APPENDIX 2: NORMAL RANGES FOR VITAL SIGNS AND ECG PARAMETERS

Vital Sign Parameters	
Pulse Rate	40-100
Systolic Blood pressure	90-140
Diastolic Blood pressure	50-90
Oral Temperature	35.0-37.5

ECG Parameters		
Heart Rate (HR)	40-100 bpm	
PR Interval	120-220 ms	
QRS Width	70-120 ms	
QT Interval	N/A	
QTc Interval - QTcB	Male: 350-430ms	Female: 350-450ms
QTc Interval - QTcF	Male: 350-450ms	Female: 350-450ms