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Protocol summary for the randomised, placebo-controlled restoration of gut microflora in critical illness trial (ROCIT)

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Abstract

Introduction

The effect of early and sustained administration of daily probiotic therapy on patients admitted to the intensive care unit (ICU) remains uncertain.

Methods and analysis

The restoration of gut microflora in critical illness trial (ROCIT) study is a multicentre, randomised, placebo-controlled, parallel-group, two-sided superiority trial that will enrol 220 patients in five ICUs. Adult patients within 48 hours of admission to an ICU and expected to require intensive care beyond the next calendar day will be randomised in a 1:1

ratio to receive early and sustained *Lactobacillus plantarum* 299v probiotic therapy in addition to usual care or placebo in addition to usual care. The primary endpoint is days alive and out of hospital to Day 60 ($DAOH_{60}$).

Ethics and dissemination

ROCIT has been approved by the South Metropolitan Health Service Human Research Ethics Committee (ref: RGS00000004) and the St John of God Health Care Human Research Ethics Committee (ref: 1183). The trial results will be submitted for publication in a peer reviewed journal.

Registration details

The trial was prospectively registered on the Australian and New Zealand Clinical Trials Registry (ANZCTR 12617000783325).

Strengths and limitations of this study

Strengths

- Early and sustained administration of study drug until determination of the primary outcome (Day-60)
- Pragmatic study design including broad eligibility criteria and administration in a usual care setting
- Sample size calculation informed by consumers and local baseline data
- Blinded adjudication of outcomes including nosocomial infection

Limitations

- A requirement to deliver and assess delivery of study drug beyond ICU and hospital discharge

Introduction

Patients admitted to the intensive care unit (ICU) commonly develop dysbiosis, an imbalance in intestinal commensal microflora characterised by a decrease in the diversity of commensal gut bacteria and an overgrowth of pathogenic species that is associated with increased morbidity and mortality [1-5]. Probiotics are live microorganisms that, when administered in adequate amounts, confer a beneficial effect on the health of the host [6]. Probiotic therapy may reduce the incidence of surgical-site infections and other post-operative complications in patients undergoing surgery [7]. In patients admitted to the ICU, probiotic therapy may reduce the risk of nosocomial infections and reduce the hospital length of stay [8-11]. A recent meta-analysis of 30 randomised controlled trials (RCTs) concluded that probiotic therapy was associated with a significant reduction in infection, but no significant effect on mortality [12]. However, study design heterogeneity and risk of bias have precluded strong recommendations for the use of probiotics in current critical care nutrition guidelines [13 14]. Furthermore, existing RCTs have generally not addressed the attributable risk of nosocomial infection, morbidity and mortality that persists after discharge from an index ICU admission.

Amongst available probiotic strains, Lp299v is a strong candidate therapy to improve outcomes in critically ill patients. Administration results in intestinal colonisation and survival of the probiotic through the entire gastrointestinal tract, regardless of gastric pH [15-17]. In otherwise healthy smokers, Lp299v therapy decreases markers of inflammation and oxidative stress [18]. In a recent landmark trial, Lp299v therapy reduced sepsis and death in rural Indian newborns [19]. In critically ill patients admitted to the ICU, Lp299v therapy exhibits similar suppression of oropharyngeal colonisation with pathogenic bacteria as chlorhexidine, reduces colonic colonisation with *Clostridiodes difficile* and attenuates

markers of systemic inflammation [20-22]. The possibility of specific benefit from Lp299v therapy in patients admitted to the ICU is supported a meta-analysis reporting that whilst probiotic therapy appears to reduce nosocomial infection in critical illness, a significant benefit is only evident in trials administering Lp299v [12].

The restoration of gut microflora in critical illness trial (ROCIT), was designed to assess whether, in adult patients admitted to the ICU, early and sustained daily administration of probiotic therapy using *Lactobacillus plantarum* 299v (Lp299v), compared with placebo, is associated with an increase in days alive and out of hospital to Day 60 ($DAOH_{60}$).

This report describes the ROCIT protocol and statistical analysis plan.

Methods and analysis

Trial design

ROCIT is a multicentre, placebo-controlled, parallel-group, two-sided superiority trial that will randomly allocate patients admitted to the ICU in a 1:1 ratio. Participants will receive probiotics in addition to usual care, or placebo in addition to usual care. ROCIT has been designed with reference to the Standard Protocol Items: Recommendations for Interval Trials checklist and is informed by consumer consultation (Consumer and Community Health Research Network, University of Western Australia, WA, 6009 [23]. The trial was prospectively registered on the Australian and New Zealand Clinical Trials Registry (ANZCTR 12617000783325).

Setting and participants

ROCIT will enrol 220 participants from five study sites in Western Australia. Eligible patients are those within 48 hours of ICU admission and who are expected to remain in the ICU beyond the next calendar day. ICU admission includes admission to a high-dependency area,

defined as an area capable of providing invasive monitoring and a nursing ratio of no greater than 1:2. Patients who will be excluded include those with an absolute contraindication to receiving medication via the enteral route, and those with one or more risk factors for treatment-associated adverse effects including recent or ongoing immunosuppressive therapy [24]. The complete inclusion and exclusion criteria are provided in Table 1. The first patient was enrolled on 28 July 2017 and recruitment to the planned sample size is expected to be completed in early 2020.

Randomisation and blinding

Eligible participants are identified by members of the study and clinical teams at participating sites. The variable-block randomisation algorithm is stratified by site and has been generated using a web-based randomisation interface by an unblinded pharmacist with no direct involvement in patient care, data collection or analysis [25]. Allocation concealment is maintained by assigning a unique number to each bottle of study drug (Figure 1). The randomisation list is kept by the unblinded pharmacist who is also available for unblinding at the request of the patient or treating team. After trial enrolment, the participant is assigned the next available subject number, corresponding to the unique, consecutively numbered bottle of study drug.

The active study drug, and the placebo are prepared in identically packaged capsules and bottles by a certified facility (Health World Ltd, 741 Nudgee Road, Northgate, Qld, 4013). All treating team members, participants, study staff and outcome adjudicators are blinded to the treatment allocation.

Study treatments

Immediately after enrolment, a dose of study drug is administered. A single capsule of the study drug is then prescribed daily, beginning the next calendar day. Instructions are

provided to continue once daily administration, including after index ICU and hospital discharge, until Day 60, (i.e. the completion of the 60-capsule bottle). A standard operating procedure (SOP) is provided to bedside clinical staff for the preparation and administration of study drug and contains instructions for nasogastric tube administration for participants unable to swallow capsules (see supplementary appendix). At the time of hospital discharge, participants are provided with a study diary to record daily study drug administration (see supplementary appendix). Participants are asked to return the completed diary along with the study drug bottle and any remaining capsules at Day 60.

Participants randomly allocated to the active study arm receive a daily capsule with $20x10^9$ colony forming units (CFUs) of Lp299v. Participants randomly allocated to the placebo arm receive an identical-looking capsule of maltodextrin. Independent batch testing of the study drug conducted by members of the study team and provided by the unblinded pharmacist confirmed $>20x10^9$ Lp299v CFU and unrecordable Lp299v CFU in the active and placebo capsules, respectively.

Study drug is transported under controlled and recorded refrigerated conditions from the manufacturer to study sites and stored under refrigerated and monitored conditions during the hospital stay. A cool bag is provided to patients for transport of the study drug on hospital discharge and patients are advised to refrigerate the study drug as soon as they arrive home. A clinical trials notification for ROCIT has been lodged with the Australian Government Therapeutic Goods Administration (ref: CT-2017-CTN-03603-1).

Concomitant therapies

Participants are requested to refrain from initiating any probiotic treatment other than the study treatment during the 60 days of study participation. Probiotics are not on the hospital

formulary of any of the five study sites participating ROCIT. All other care is at the discretion of the treating teams.

Outcomes

The flow of participants in the study will be reported according to CONSORT criteria (Figure 2)[26]. The primary outcome is days alive and out of hospital ($DAOH_{60}$). DAOH is a validated measure that includes death, length of stay (LOS) in hospital, need for ongoing rehabilitation and the occurrence and duration of hospital readmission [27-30]. Days spent in a rehabilitation facility or high-level nursing facility to Day 60 are considered as days in hospital. Participants who die prior to Day 60 will be recorded as having zero $DAOH_{60}$ [31].

Secondary endpoints include the occurrence of specified nosocomial infections (hospital-acquired pneumonia, ventilator-associated pneumonia, *C. difficile*-associated diarrhoea, surgical site infection, urinary tract infection, and blood stream infection) defined according to Centre for Disease Control (CDC) criteria (supplementary appendix) [32]. Screening for nosocomial infection will occur by identifying each episode of initiation or change of antibiotic to Day-60 and will then be assessed independently by two blinded infectious diseases specialist clinicians by review of the medical records. Any disagreement will be resolved by consensus. Other secondary endpoints include antibiotic-free days to Day 60, ICU and hospital LOS, and ICU, hospital and 60-day mortality. Quality of life will be assessed using the EQ 5D-5L at Day 60, administered via telephone by blinded research staff at each study site (Table 2).

Data collection and management

Trained research coordinators will collect data at each site using a study-specific case report form. Study data are entered into a REDCap database, a secure, web-based software platform [33]. Assessment of the primary outcome will include direct phone contact with

participants on or shortly after Day 60 where participants are not known to be hospitalised or have died. Details of the occurrence and duration of an hospital readmissions will be collected during this phone call and cross-checked against hospital medical records, and, if required, general practitioner records. To ensure the accuracy and completeness of data there will be pre-specified automatic checks and on-site data monitoring by the project manager, including 100% source data verification for the primary endpoint. Screening, baseline, daily, outcome, adverse event and protocol deviation data are provided in Table 2.

Sample size and power

A difference of four days in $DAOH_{60}$ is considered meaningful by a specially convened forum of consumers including ICU survivors and next-of-kin (Consumer and Community Health Research Network, University of Western Australia, WA, 6009). Baseline $DAOH_{60}$ has been calculated using contemporary data from participating hospitals. From these data, a baseline of 37 $DAOH_{60}$, a standard deviation (SD) of nine, and a two-tailed α=0.05, a trial of 162 participants has 80% power to detect a difference in $DAOH_{60}$ of four days. After inflation for non-normal distribution (20%), withdrawn consent (5%) and loss to follow up (5%), the final sample size is 220 participants.

Statistical analysis plan

The primary analysis will be the intention-to-treat population, defined as all eligible and randomised study participants, except for those who do not consent to use of the data necessary to determine the primary outcome. There will be no imputation for missing data. Normally distributed data will be presented as mean (SD), and non-normally distributed data as median (interquartile range [IQR]). Comparisons will be performed using Fisher's exact test for categorical data, and Student t test or Wilcoxon rank sum test for normally and non-normally distributed data respectively. The primary outcome ($DAOH_{60}$), will be

analysed using Wilcoxon rank sum test with results presented as a comparison of medians (IQR). A two-sided P-value of <0.05 will be considered statistically significant. Heterogeneity between pre-specified subgroups, identified at baseline, will be assessed by fitting an interaction term between treatment and subgroup.

The three subgroup pairs will be: patients with sepsis versus those without sepsis; emergency versus elective ICU admissions; and surgical versus medical admissions. A per-protocol analysis will be conducted including all participants with reported adherence to the study medication for >80% of their total study duration. Planned sub-studies include longitudinal evaluation of faecal microbiome and blood metabolome, and, if there is statistically significant difference in the primary outcome, an economic evaluation of the cost-effectiveness of the intervention. All analyses will be conducted using STATA/SE 13 (College Station, TX, USA).

Patient and public involvement

The primary outcome was chosen on the basis of published evidence of the importance placed by patients on days spent at home[34]. Consideration of additional outcome measures was made in conjunction with an ICU consumer forum convened from the Consumer and Community Health Research Network (University of Western Australia, WA, 6009). Study participants are offered the opportunity to have the published study results supplied to them directly and to be unblinded after final determination of all study outcome measures. The published manuscript of the primary outcome will be made available to the Consumer and Community Health Research Network for dissemination amongst stakeholders.

Data monitoring committee

The data monitoring committee (DMC) has expertise in critical care, infectious diseases and trial design but are not otherwise involved in the care of study participants. The members

are Nolan McDonnell BHB MBChB FANZCA MClinRes, Claire Italiano MBBS FRACP MPHTM, and Ravi Sonowane MBBS FCICM MPH. The DMC has reviewed and approved the study protocol and will review all serious adverse events as they occur. The ROCIT management committee will inform the DMC of any accumulating external evidence of relevance to the ongoing conduct of the study as soon as practicable. No interim analyses are planned but the DMC will reserve the right to conduct an interim analysis, or advise suspension or termination of ongoing enrolment to the study.

Adverse events

Events that are a part of the natural history of the primary disease process or expected complication of critical illness will not be reported as serious adverse events [35]. All adverse events considered to be potentially causally related to the trial will be reported.

Ethics and dissemination

Ethics approval

ROCIT has been approved by the South Metropolitan Health Service Human Research Ethics Committee (ref: RGS00000004) and the St John of God Health Care Human Research Ethics Committee (ref: 1183). The approved consent pathways included prospective participant consent for study-eligible patients with capacity, and prospective Person Responsible acknowledgement with deferred consent for patients who lacked capacity.

Dissemination

The study results will be submitted for publication in a peer reviewed journal. Study data and statistical code can be accessed by contacting the corresponding author. Requests for access will be reviewed by the named authors on a case-by-case basis.

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Author contribution

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Competing interest

The authors declare no competing interests.

Table 1 Trial eligibility criteria

Inclusion criteria	1. Adult patient within 48 hours of admission to an ICU

	2. Expected to require ICU-level care beyond the next calendar day
Exclusion criteria	1. <18 years of age 2. Absolute contraindication to receiving medication via the enteral route 3. Known to be receiving probiotic therapy at the time of index hospitalisation 4. Acute pancreatitis as a cause or complication of current admission 5. Immunosuppression (defined as chemotherapy within the preceding four weeks, or receiving ≧ 1.5mg/kg methylprednisolone daily or equivalent) 6. Neutropenia (neutrophil count ≦ $1x10^9$/L) 7. Prosthetic heart valve or permanent pacemaker 8. Death is deemed to be inevitable as a result of the current acute illness AND either the treating clinician, the patient or the substitute decision-maker, are not committed to full active treatment 9. Enrolment is not considered in the patient's best interest 10. Previously enrolled in ROCIT 11. Unlikely to be residing near or visiting a study centre in 60 days 12. Participating in a competing interventional study 13. Pregnancy 14. Admitted to hospital from a high-level nursing facility or rehabilitation facility

ICU intensive care unit, ROCIT restoration of gut microflora in critical illness trial

Table 2. Study data to be collected

Time point	Study data
Screening	- Date of screening - Inclusion and exclusion criteria - Reason, if not enrolled - Study number, patient initials, for enrolled participants
Baseline	- Date and time of randomisation - Date and time of ICU admission - Demographic data - ICU admission source and category - Nutrition, acid suppressive therapy and antibiotics - Admission APACHE II score, diagnostic code and co-morbidities - SOFA Score and components - Mechanical ventilation

	- Vasoactive medication - Renal replacement therapy
Daily during index hospitalisation	- Patient location (ICU/HDA or ward) - Received study drug - Days of mechanical ventilation, vasoactive medication and renal replacement therapy - Days of antibiotic, antiviral and antifungal medication - New infection diagnosed
Outcome (Day 60)	- Hospital length of stay - Nosocomial infection (hospital-acquired pneumonia, ventilator-associated pneumonia, Clostridium difficile-associated diarrhoea, surgical site infection, urinary tract infection, and blood stream infection)* - ICU length of stay - ICU mortality - Hospital mortality - EQ-5D-5L
Adverse events	- Description, timing, causality and resolution of adverse events from randomisation to Day 60
Protocol deviations	- Randomisation of ineligible patients, failure to comply with the study protocol

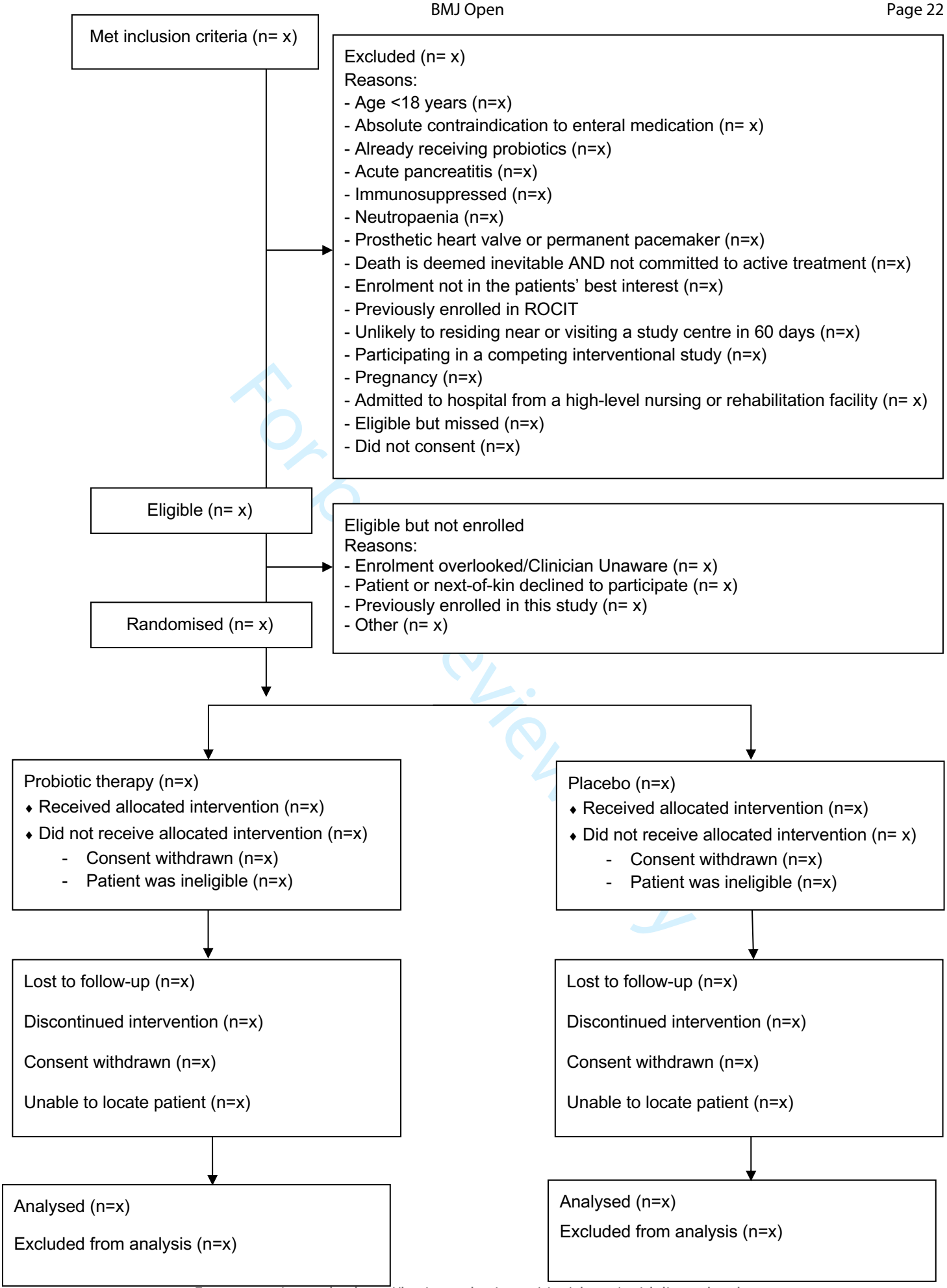
ICU intensive care unit, HDA High dependency area, APACHE acute physiology and chronic health evaluation, SOFA Sequential organ failure score, EQ-5D-5L five level EuroQol five-dimension questionnaire.
*The pre-specified nosocomial infections will be identified according to the centre for disease control definitions and are provided in the supplementary appendix

Figure legends

Figure 1. Study drug bottle labelling

Figure 2. Proposed reporting of flow of trial participants

Manufacturer: Health World Limited 741 Nudgee Road, Northgate QLD 4013 Qld Tel: 07 3117 3300 Sponsor: Fiona Stanley Hospital, 11 Robin Warren Drive, Murdoch WA 6150 PI: Dr Ed Litton 08 6152 2222 ONCE YOU HAVE COMPLETED TREATMENT PLEASE RETURN ALL PARTIALLY USED AND EMPTY BOTTLES TO THE PHARMACY DEPARTMENT OF YOUR TREATING HOSPITAL Store at 2-8°C (do not freeze)	Keep out of reach of children For clinical trial use only / For oral use only ROCIT trial ANZCTRN: 12617000783325 PROBIOTIC Lactobacillus plantarum 299V 20 billion CFU or PLACEBO capsule (60 capsules) Take ONE capsule each day with a glass of water until all capsules have been taken. Bottle Number: XXXX Subject ID: XXXX Subject Name: _____ UMRN: _____ Date Dispensed: _____ Batch Number: Expiry:



ROCIT Protocol Supplementary Appendix

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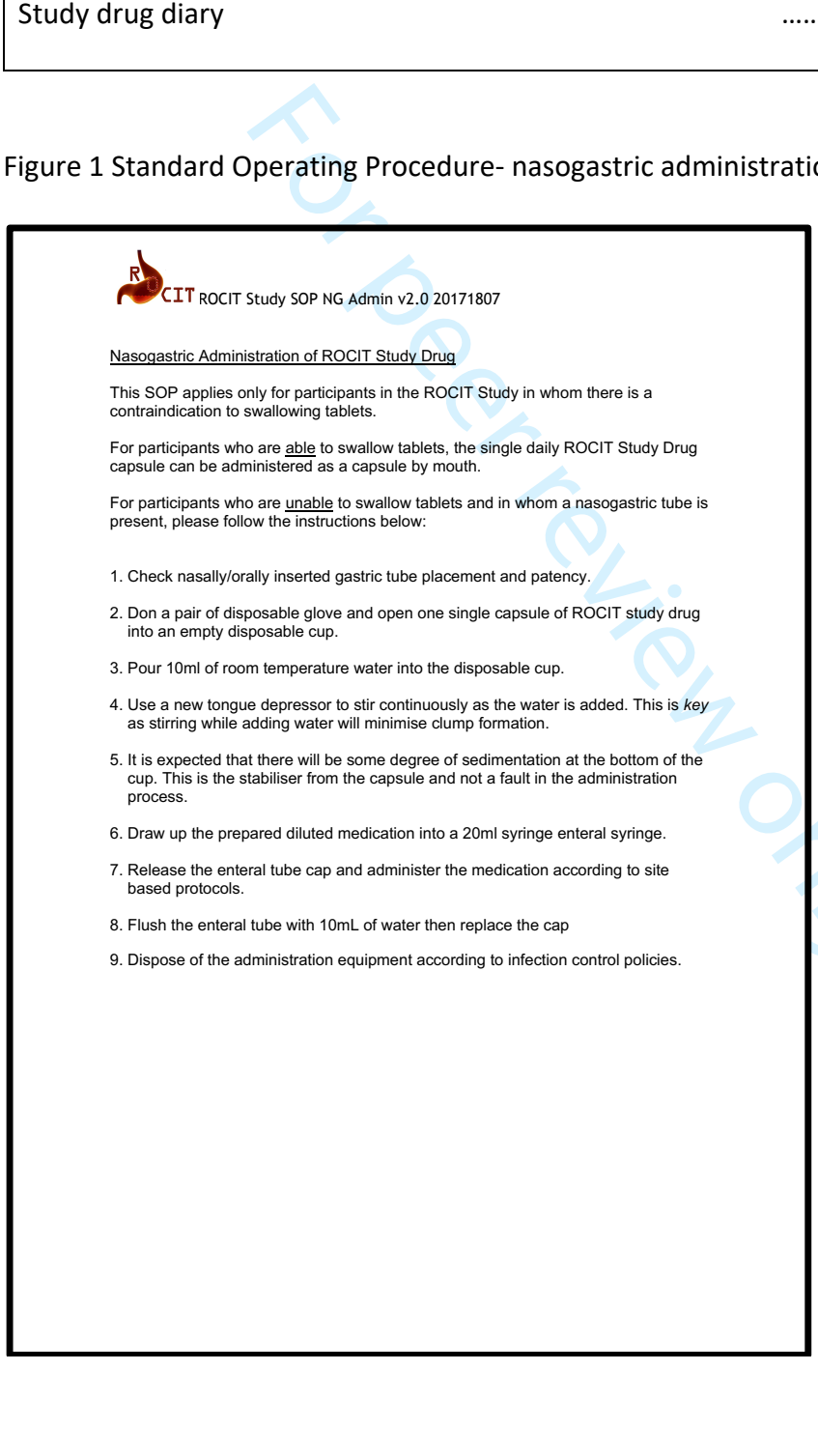
Study drug diary	.....	4

Figure 1 Standard Operating Procedure- nasogastric administration



ROCIT Study SOP NG Admin v2.0 20171807

Nasogastric Administration of ROCIT Study Drug

This SOP applies only for participants in the ROCIT Study in whom there is a contraindication to swallowing tablets.

For participants who are able to swallow tablets, the single daily ROCIT Study Drug capsule can be administered as a capsule by mouth.

For participants who are unable to swallow tablets and in whom a nasogastric tube is present, please follow the instructions below:

1. Check nasally/orally inserted gastric tube placement and patency.
2. Don a pair of disposable glove and open one single capsule of ROCIT study drug into an empty disposable cup.
3. Pour 10ml of room temperature water into the disposable cup.
4. Use a new tongue depressor to stir continuously as the water is added. This is *key* as stirring while adding water will minimise clump formation.
5. It is expected that there will be some degree of sedimentation at the bottom of the cup. This is the stabiliser from the capsule and not a fault in the administration process.
6. Draw up the prepared diluted medication into a 20ml syringe enteral syringe.
7. Release the enteral tube cap and administer the medication according to site based protocols.
8. Flush the enteral tube with 10mL of water then replace the cap
9. Dispose of the administration equipment according to infection control policies.

Table 1 Summary (abbreviated) of nosocomial infection diagnostic criteria*

Nosocomial infection	Criteria
Surgical site infection	Superficial incisional: occurs within 30 days after the operation and the infection involves only skin or subcutaneous tissue of the incision and at least one of the following: 1. Purulent drainage 2. Organisms isolated 3. At least one of pain, tenderness, swelling, redness, heat and superficial incision is deliberately opened by surgeon 4. Diagnosis of superficial incisional by surgeon Deep incisional: occurs within 30 days after the operation if no implant or within 1 year if implant and related to the operation and infection involves the deep tissues and at least one of the following: 1. Purulent drainage from the deep incision, but not from the organ of the surgical site 2. Deep incision spontaneously dehisces or is deliberately opened by the surgeon 3. Abscess or other evidence of infection involving the deep incision 4. Diagnosis of deep surgical space infection by the surgeon Organ/space: occurs within 30 days or within one year if implant is in place and related to the operation and infection involves any part of the anatomy other than the incision and: 1. Purulent drainage from a drain into the organ space 2. Organisms isolated aseptically from the organ/space 3. Abscess from the organ/space 4. Diagnosis of an organ/space infection by the surgeon The CDC criteria for burns infection will be included as a surgical site infection.
Blood stream infection	Clinical criteria are only required if the organism identified is a common skin contaminant Clinical criteria (at least one of): 1. Fever 2. WBC>10,000 or <3,000 per cubic millimetre 3. Hypotension (SBP<90) or >25% drop in SBP

C. difficile-associated diarrhoea	Clinical evidence of at least one of the following: 1. Pseudomembranes identified at lower gastrointestinal endoscopy 2. Pathological confirmation of pseudomembranous colitis 3. C difficile toxin detected in the stool
Urinary tract infection	Clinical criteria of a and b must be satisfied within a two-day period a) Clinical criteria (at least one of): i. fever >38.5 C ii. WBC>10,000 or <3,000 per cubic millimetre iii. Urgency iv. Dysuria v. Suprapubic tenderness b) Bacterial confirmation vi. $>10^5$ organisms per ml of urine
Pneumonia	Criteria a-c must be satisfied within a 48-hour period a) Radiologic criteria i. New infiltrate that persists for at least 24 hours b) Clinical criteria (one of) i. Temperature >38.5 or <35 C ii. WBC>10,000 or <3,000 per cubic millimetre c) Bacterial confirmation (at least one of) i. BAL $>10^3$ CFU/ml ii. Histopathological examination of lung tissue iii. Positive blood culture for bacterial pathogen identified in sputum iv. Positive pleural culture with same organism in sputum v. Positive gram stain vi. Heavy or moderate growth of one type of pathogenic bacteria on semi-quantitative sputum culture Ventilator-associated pneumonia occurs where a patient is on mechanical ventilation for > two calendar days on the date of the event, with day of ventilator placement being day one, and the ventilator was in place on the date of the event or the day before.

WBC white blood cell, SBP systolic blood pressure, BAL bronchoalveolar lavage, CFU colony forming units. *For full criteria and wording see CDC. National Healthcare Safety Network (NHSN) Patient Safety Component Manual. Chapter 17: CDC/NHSN Surveillance Definitions for Specific Types of Infections. 2019. Where any difference occurs between this summary and the CDC definitions, the CDC definitions will take priority.

Figure 2. Study drug diary

ROCIT Study daily medication diary

1□	2□	3□	4□	5□	6□
7□	8□	9□	10□	11□	12□
13□	14□	15□	16□	17□	18□
19□	20□	21□	22□	23□	24□
25□	26□	27□	28□	29□	30□

Date of Hospital Discharge: Participant #:

Please record daily probiotics taken by ticking the box and make any comments on the day in the space provided.
Please keep and return the study bottles.
Any questions please contact the Dr Ed Litton or the Research Coordinators **via FSH switch board on 615 22222**

ROCIT Study daily medication diary

31□	32□	33□	34□	35□	36□
37□	38□	39□	40□	41□	42□
43□	44□	45□	46□	47□	48□
49□	50□	51□	52□	53□	54□
55□	56□	57□	58□	59□	60□

Please record daily probiotics taken by ticking the box and make any comments on the day in the space provided.
Please keep and return the study bottle.
Any questions please contact the Dr Ed Litton or the Research Coordinators via FSH switch board on 615 2222

BMJ Open

Study protocol for the safety and efficacy of probiotic therapy on days alive and out of hospital in adult ICU patients: The multi-centre, randomised, placebo-controlled restoration of gut microflora in critical illness trial (ROCIT).

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Study protocol for the safety and efficacy of probiotic therapy on days alive and out of hospital in adult ICU patients: The multi-centre, randomised, placebo-controlled restoration of gut microflora in critical illness trial (ROCIT).

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Abstract

Introduction

The effect of early and sustained administration of daily probiotic therapy on patients admitted to the intensive care unit (ICU) remains uncertain.

Methods and analysis

The restoration of gut microflora in critical illness trial (ROCIT) study is a multicentre, randomised, placebo-controlled, parallel-group, two-sided superiority trial that will enrol 220 patients in five ICUs. Adult patients within 48 hours of admission to an ICU and

expected to require intensive care beyond the next calendar day will be randomised in a 1:1 ratio to receive early and sustained *Lactobacillus plantarum* 299v probiotic therapy in addition to usual care or placebo in addition to usual care. The primary endpoint is days alive and out of hospital to Day 60 ($DAOH_{60}$).

Ethics and dissemination

ROCIT has been approved by the South Metropolitan Health Service Human Research Ethics Committee (ref: RGS00000004) and the St John of God Health Care Human Research Ethics Committee (ref: 1183). The trial results will be submitted for publication in a peer reviewed journal.

Registration details

The trial was prospectively registered on the Australian and New Zealand Clinical Trials Registry (ANZCTR 12617000783325).

Strengths and limitations of this study

Strengths

- Early and sustained administration of study drug until determination of the primary outcome (Day-60)
- Pragmatic study design including broad eligibility criteria and administration in a usual care setting
- Sample size calculation informed by consumers and local baseline data
- Blinded adjudication of outcomes including nosocomial infection

Limitations

- A requirement to deliver and assess delivery of study drug beyond ICU and hospital discharge

Introduction

Patients admitted to the intensive care unit (ICU) commonly develop dysbiosis, an imbalance in intestinal commensal microflora characterised by a decrease in the diversity of commensal gut bacteria and an overgrowth of pathogenic species that is associated with increased morbidity and mortality [1-5]. Probiotics are live microorganisms that, when administered in adequate amounts, confer a beneficial effect on the health of the host [6]. Probiotic therapy may reduce the incidence of surgical-site infections and other post-operative complications in patients undergoing surgery [7]. In patients admitted to the ICU, probiotic therapy may reduce the risk of nosocomial infections and reduce the hospital length of stay [8-11]. A recent meta-analysis of 30 randomised controlled trials (RCTs) concluded that probiotic therapy was associated with a significant reduction in infection, but no significant effect on mortality [12]. However, study design heterogeneity and risk of bias have precluded strong recommendations for the use of probiotics in current critical care nutrition guidelines [13 14]. Furthermore, existing RCTs have generally not addressed the attributable risk of nosocomial infection, morbidity and mortality that persists after discharge from an index ICU admission[15].

Amongst available probiotic strains, Lp299v is a strong candidate therapy to improve outcomes in critically ill patients. Administration results in intestinal colonisation and survival of the probiotic through the entire gastrointestinal tract, regardless of gastric pH [16-18]. In otherwise healthy smokers, Lp299v therapy decreases markers of inflammation and oxidative stress [19]. In a recent landmark trial, Lp299v therapy reduced sepsis and death in rural Indian newborns [20]. In critically ill patients admitted to the ICU, Lp299v therapy exhibits similar suppression of oropharyngeal colonisation with pathogenic bacteria as

chlorhexidine, reduces colonic colonisation with *Clostridiodes difficile* and attenuates markers of systemic inflammation [21-23]. The possibility of specific benefit from Lp299v therapy in patients admitted to the ICU is supported by a meta-analysis reporting that whilst probiotic therapy appears to reduce nosocomial infection in critical illness, a significant benefit is only evident in trials administering Lp299v [12]. However, recent evidence suggests that probiotic Lactobacilli strains can directly cause bacteraemia when administered to patients in ICU and the safety and efficacy of Lp229v in adult patients admitted to the ICU remains uncertain[24].

The restoration of gut microflora in critical illness trial (ROCIT), was designed to assess whether, in adult patients admitted to the ICU, early and sustained daily administration of probiotic therapy using *Lactobacillus plantarum* 299v (Lp299v), compared with placebo, is associated with an increase in days alive and out of hospital to Day 60 ($DAOH_{60}$).

This report describes the ROCIT protocol and statistical analysis plan.

Methods and analysis

Trial design

ROCIT is a multicentre, placebo-controlled, parallel-group, two-sided superiority trial that will randomly allocate patients admitted to the ICU in a 1:1 ratio. Participants will receive probiotics in addition to usual care, or placebo in addition to usual care. ROCIT has been designed with reference to the Standard Protocol Items: Recommendations for Interval Trials checklist and is informed by consumer consultation (Consumer and Community Health Research Network, University of Western Australia, WA, 6009 [25]. The trial was prospectively registered on the Australian and New Zealand Clinical Trials Registry (ANZCTR 12617000783325).

Setting and participants

ROCIT will enrol 220 participants from five study sites in Western Australia (see supplementary appendix for the study site list). Eligible patients are those within 48 hours of ICU admission and who are expected to remain in the ICU beyond the next calendar day. ICU admission includes admission to a high-dependency area, defined as an area capable of providing invasive monitoring and a nursing ratio of no greater than 1:2. Patients who will be excluded include those with an absolute contraindication to receiving medication via the enteral route, and those with one or more risk factors for treatment-associated adverse effects including recent or ongoing immunosuppressive therapy [26]. The complete inclusion and exclusion criteria are provided in Table 1. The first patient was enrolled on 28 July 2017 and recruitment to the planned sample size is expected to be completed in early 2020.

Randomisation and blinding

Eligible participants are identified by members of the study and clinical teams at participating sites. This pragmatic approach, embedded in clinical care, will maximise recruitment. The variable-block randomisation algorithm is stratified by site and has been generated using a web-based randomisation interface by an unblinded pharmacist with no direct involvement in patient care, data collection or analysis [27]. Allocation concealment is maintained by assigning a unique number to each bottle of study drug (Figure 1). The randomisation list is kept by the unblinded pharmacist who is also available for unblinding at the request of the patient or treating team. After trial enrolment, the participant is assigned the next available subject number, corresponding to the unique, consecutively numbered bottle of study drug.

The active study drug, and the placebo are prepared in identically packaged capsules and bottles by a certified facility (Health World Ltd, 741 Nudgee Road, Northgate, Qld, 4013). All

treating team members, participants, study staff and outcome adjudicators are blinded to the treatment allocation.

Study treatments

Immediately after enrolment, a dose of study drug is administered. A single capsule of the study drug is then prescribed daily, beginning the next calendar day. Instructions are provided to continue once daily administration, including after index ICU and hospital discharge, until Day 60, (i.e. the completion of the 60-capsule bottle). A standard operating procedure (SOP) is provided to bedside clinical staff for the preparation and administration of study drug and contains instructions for nasogastric tube administration for participants unable to swallow capsules (see Supplementary Appendix Figure 1.). At the time of hospital discharge, participants are provided with a study diary to record daily study drug administration (see Supplementary Appendix Figure 2.). Participants are asked to return the completed diary along with the study drug bottle and any remaining capsules at Day 60. Participants randomly allocated to the active study arm receive a daily capsule with $20x10^9$ colony forming units (CFUs) of Lp299v. Participants randomly allocated to the placebo arm receive an identical-looking capsule of maltodextrin. Independent batch testing of the study drug conducted by members of the study team and provided by the unblinded pharmacist confirmed $>20x10^9$ Lp299v CFU and unrecordable Lp299v CFU in the active and placebo capsules, respectively.

Study drug is transported under controlled and recorded refrigerated conditions from the manufacturer to study sites and stored under refrigerated and monitored conditions during the hospital stay. A cool bag is provided to patients for transport of the study drug on hospital discharge and patients are advised to refrigerate the study drug as soon as they

arrive home. A clinical trials notification for ROCIT has been lodged with the Australian Government Therapeutic Goods Administration (ref: CT-2017-CTN-03603-1).

Concomitant therapies

Participants are requested to refrain from initiating any probiotic treatment other than the study treatment during the 60 days of study participation. Probiotics are not on the hospital formulary of any of the five study sites participating ROCIT. All other care is at the discretion of the treating teams.

Discontinuation

Study drug may be discontinued at the request of the participant or treating clinician at any stage if the participant or treating clinician suspects an adverse reaction or that continued participation is not in the best interest of the participant. A suspected or confirmed severe adverse drug reaction will result in immediate and permanent discontinuation of the study medication. Study drug will also be discontinued permanently if Lactobacillus Plantarum is grown from a sterile site or is the predominant growth from a non-sterile site.

Outcomes

The flow of participants in the study will be reported according to CONSORT criteria (Figure 2)[28]. The primary outcome is days alive and out of hospital ($DAOH_{60}$). DAOH is a validated measure that includes death, length of stay (LOS) in hospital, need for ongoing rehabilitation and the occurrence and duration of hospital readmission [29-32]. Days spent in a rehabilitation facility or high-level nursing facility to Day 60 are considered as days in hospital. Participants who die prior to Day 60 will be recorded as having zero $DAOH_{60}$ [33].

Secondary endpoints include the occurrence of specified nosocomial infections (hospital-acquired pneumonia, ventilator-associated pneumonia, *C. difficile*-associated diarrhoea, surgical site infection, urinary tract infection, and blood stream infection) defined according

to Centre for Disease Control (CDC) criteria (see Supplementary Appendix Table 1.) [34]. Screening for nosocomial infection will occur by identifying each episode of initiation or change of antibiotic to Day 60 and will then be assessed independently by two blinded infectious diseases specialist clinicians by review of the medical records. Any disagreement will be resolved by consensus. Other secondary endpoints include antibiotic-free days to Day 60, ICU and hospital LOS, and ICU, hospital and 60-day mortality. Quality of life will be assessed using the five-level EuroQol (EQ 5D-5L) at Day 60, administered via telephone by blinded research staff at each study site (Table 2).

Data collection and management

Trained research coordinators will collect data at each site using a study-specific case report form. Study data are entered into a REDCap database, a secure, web-based software platform [35]. Assessment of the primary outcome will include direct phone contact with participants on or shortly after Day 60 where participants are not known to be hospitalised or have died. Details of the occurrence and duration of an hospital readmissions will be collected during this phone call and cross-checked against hospital medical records, and, if required, general practitioner records. To ensure the accuracy and completeness of data there will be pre-specified automatic checks and on-site data monitoring by the project manager, including 100% source data verification for the primary endpoint. Screening, baseline, daily, outcome, adverse event and protocol deviation data are provided in Table 2. The plans for collecting and storing biological specimens for analysis in ancillary studies are provided in the supplementary appendix.

Sample size and power

A difference of four days in $DAOH_{60}$ is considered meaningful by a specially convened forum of consumers including ICU survivors and next-of-kin (Consumer and Community Health

Research Network, University of Western Australia, WA, 6009). Baseline $DAOH_{60}$ has been calculated using contemporary data from participating hospitals. From these data, a baseline of 37 $DAOH_{60}$, a standard deviation (SD) of nine, and a two-tailed α=0.05, a trial of 162 participants has 80% power to detect a difference in $DAOH_{60}$ of four days. After inflation for non-normal distribution (20%), withdrawn consent (5%) and loss to follow up (5%), the final sample size is 220 participants.

Statistical analysis plan

The primary analysis will be the intention-to-treat population, defined as all eligible and randomised study participants, except for those who do not consent to use of the data necessary to determine the primary outcome. There will be no imputation for missing data. Normally distributed data will be presented as mean (SD), and non-normally distributed data as median (interquartile range [IQR]). Comparisons will be performed using Fisher's exact test for categorical data, and Student t test or Wilcoxon rank sum test for normally and non-normally distributed data respectively. The primary outcome ($DAOH_{60}$), will be analysed using Wilcoxon rank sum test with results presented as a comparison of medians (IQR). A two-sided P-value of <0.05 will be considered statistically significant. Heterogeneity between pre-specified subgroups, identified at baseline, will be assessed by fitting an interaction term between treatment and subgroup.

The three subgroup pairs will be: patients with sepsis versus those without sepsis; emergency versus elective ICU admissions; and surgical versus medical admissions. A per-protocol analysis will be conducted including all participants with reported adherence to the study medication for >80% of their total study duration. Planned sub-studies include longitudinal evaluation of faecal microbiome and blood metabolome, and, if there is statistically significant difference in the primary outcome, an economic evaluation of the

cost-effectiveness of the intervention. All analyses will be conducted using STATA/SE 13 (College Station, TX, USA).

Patient and public involvement

The primary outcome was chosen on the basis of published evidence of the importance placed by patients on days spent at home[36]. Consideration of additional outcome measures was made in conjunction with an ICU consumer forum convened from the Consumer and Community Health Research Network (University of Western Australia, WA, 6009). Study participants are offered the opportunity to have the published study results supplied to them directly and to be unblinded after final determination of all study outcome measures. The published manuscript of the primary outcome will be made available to the Consumer and Community Health Research Network for dissemination amongst stakeholders.

Data monitoring committee

The data monitoring committee (DMC) has expertise in critical care, infectious diseases and trial design but are not otherwise involved in the care of study participants and is independent from competing interests. The members are Nolan McDonnell BHB MBChB FANZCA MClinRes, Claire Italiano MBBS FRACP MPHTM, and Ravi Sonowane MBBS FCICM MPH. The DMC has reviewed and approved the study protocol and will review all serious adverse events as they occur. The ROCIT management committee will inform the DMC of any accumulating external evidence of relevance to the ongoing conduct of the study as soon as practicable. No interim analyses are planned but the DMC will reserve the right to conduct an interim analysis, or advise suspension or termination of ongoing enrolment to the study.

Adverse events

Events that are a part of the natural history of the primary disease process or expected complication of critical illness will not be reported as serious adverse events [37]. All adverse events considered to be potentially causally related to the trial, and all serious adverse events, will be reported (supplementary appendix Table 2).

Ethics and dissemination

Ethics approval

ROCIT has been approved by the South Metropolitan Health Service Human Research Ethics Committee (ref: RGS00000004) and the St John of God Health Care Human Research Ethics Committee (ref: 1183). The approved consent pathways included prospective participant consent for study-eligible patients with capacity, and prospective Person Responsible acknowledgement with deferred consent for patients who lacked capacity. Protocol modifications will be submitted to Human Research Ethics Committee review prior to dissemination and initiation at trial sites. A copy of the consent form is provided in the supplementary appendix.

Dissemination

The study results will be submitted for publication in a peer reviewed journal. Study data and statistical code can be accessed by contacting the corresponding author. Requests for access will be reviewed by the named authors on a case-by-case basis.

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Author contribution

Edward Litton has made a substantial contribution to the design of the work and drafting the work and has given final approval for the work and agrees to be accountable for all aspects of the work, Matt Anstey has made a substantial contribution to the design of the work and revising the work and has given final approval for the work and agrees to be accountable for all aspects of the work, David Broadhurst has made a substantial contribution to the design of the work and revising the work and has given final approval for the work and agrees to be accountable for all aspects of the work, Andy Chapman has made a substantial contribution to the design of the work and revising the work and has given final approval for the work and agrees to be accountable for all aspects of the work, Andrew Currie has made a substantial contribution to the design of the work and revising the work and has given final approval for the work and agrees to be accountable for all aspects of the work, Janet Ferrier has made a substantial contribution to the design of the work and revising the work and has given final approval for the work and agrees to be accountable for all aspects of the work, Joel Gummer has made a substantial contribution to the design of the work and revising the work and has given final approval for the work and agrees to be accountable for all aspects of the work, Alisa Higgins has made a substantial contribution to the design of the work and revising the work and has given final approval for the work and agrees to be accountable for all aspects of the work, Jolene Lim has made a substantial contribution to the design of the work and revising the work and has given final approval for the work and agrees to be accountable for all aspects of the work, Laurens Manning has made a substantial contribution to the design of the work and revising the work and has given final approval for the work and agrees to be accountable for all aspects of the work, Erina Myer has made a substantial contribution to the design of the work and revising the work and has given final approval for the work and agrees to be accountable for all aspects

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Competing interest

The authors declare no competing interests.

Table 1 Trial eligibility criteria

Inclusion criteria	1. Adult patient within 48 hours of admission to an ICU

	2. Expected to require ICU-level care beyond the next calendar day
Exclusion criteria	1. <18 years of age 2. Absolute contraindication to receiving medication via the enteral route 3. Known to be receiving probiotic therapy at the time of index hospitalisation 4. Acute pancreatitis as a cause or complication of current admission 5. Immunosuppression (defined as chemotherapy within the preceding four weeks, or receiving ≧ 1.5mg/kg methylprednisolone daily or equivalent) 6. Neutropenia (neutrophil count ≦ $1x10^9$/L) 7. Prosthetic heart valve or permanent pacemaker 8. Death is deemed to be inevitable as a result of the current acute illness AND either the treating clinician, the patient or the substitute decision-maker, are not committed to full active treatment 9. Enrolment is not considered in the patient's best interest 10. Previously enrolled in ROCIT 11. Unlikely to be residing near or visiting a study centre in 60 days 12. Participating in a competing interventional study 13. Pregnancy 14. Admitted to hospital from a high-level nursing facility or rehabilitation facility

ICU intensive care unit, ROCIT restoration of gut microflora in critical illness trial

Table 2. Study data to be collected

Time point	Study data
Screening	- Date of screening - Inclusion and exclusion criteria - Reason, if not enrolled - Study number, patient initials, for enrolled participants
Baseline	- Date and time of randomisation - Date and time of ICU admission - Demographic data - ICU admission source and category - Nutrition, acid suppressive therapy and antibiotics - Admission APACHE II score, diagnostic code and co-morbidities - SOFA Score and components - Mechanical ventilation

	- Vasoactive medication - Renal replacement therapy
Daily during index hospitalisation	- Patient location (ICU/HDA or ward) - Received study drug - Days of mechanical ventilation, vasoactive medication and renal replacement therapy - Days of antibiotic, antiviral and antifungal medication - New infection diagnosed
Outcome (Day 60)	- Hospital length of stay - Nosocomial infection (hospital-acquired pneumonia, ventilator-associated pneumonia, Clostridium difficile-associated diarrhoea, surgical site infection, urinary tract infection, and blood stream infection)* - ICU length of stay - ICU mortality - Hospital mortality - EQ-5D-5L
Adverse events	- Description, timing, causality and resolution of adverse events from randomisation to Day 60
Protocol deviations	- Randomisation of ineligible patients, failure to comply with the study protocol

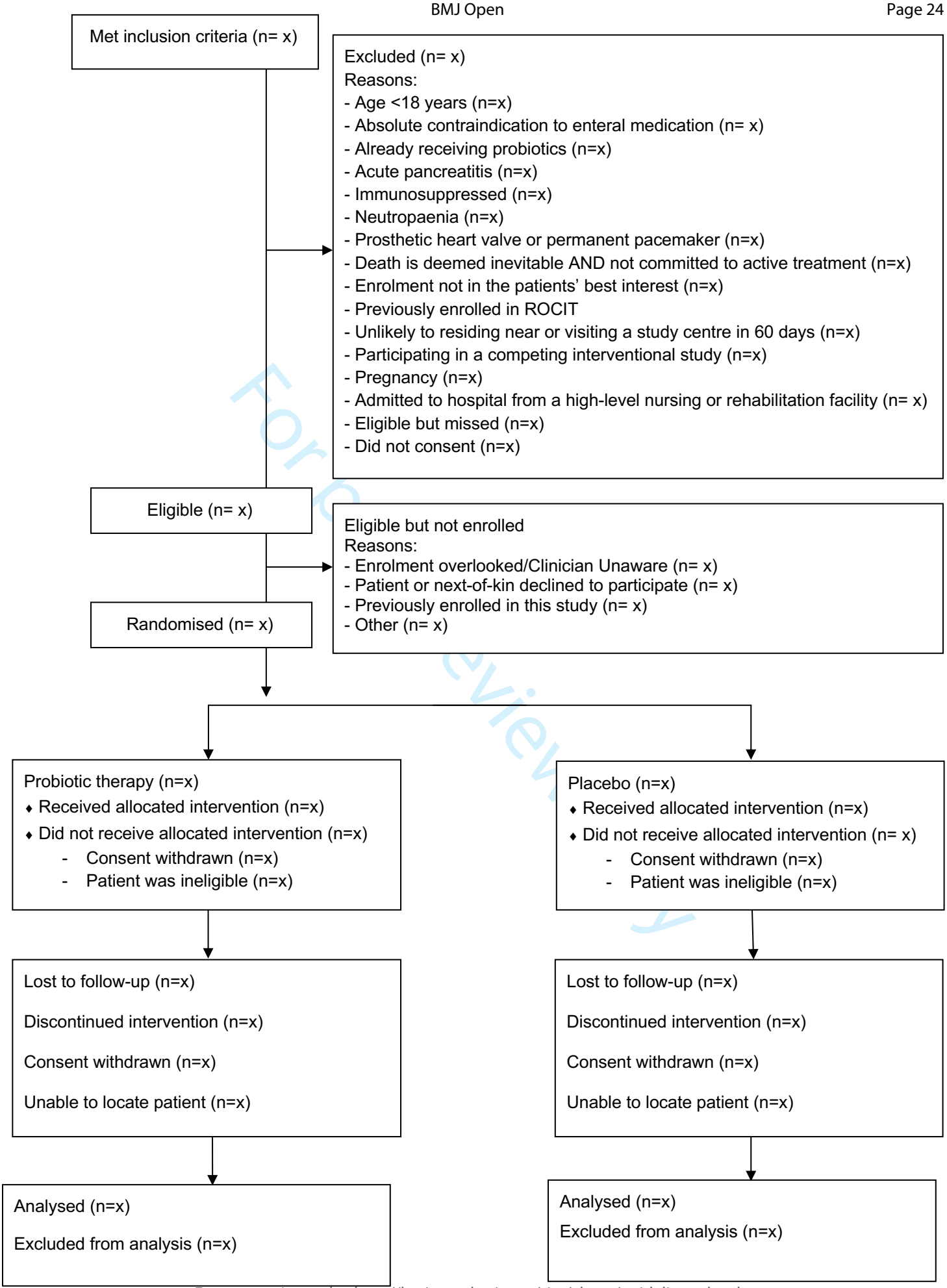
ICU intensive care unit, HDA High dependency area, APACHE acute physiology and chronic health evaluation, SOFA Sequential organ failure score, EQ-5D-5L five level EuroQol five-dimension questionnaire.
*The pre-specified nosocomial infections will be identified according to the centre for disease control definitions and are provided in the supplementary appendix

Figure legends

Figure 1. Study drug bottle labelling

Figure 2. Proposed reporting of flow of trial participants

Manufacturer: Health World Limited 741 Nudgee Road, Northgate QLD 4013 Qld Tel: 07 3117 3300 Sponsor: Fiona Stanley Hospital, 11 Robin Warren Drive, Murdoch WA 6150 PI: Dr Ed Litton 08 6152 2222 ONCE YOU HAVE COMPLETED TREATMENT PLEASE RETURN ALL PARTIALLY USED AND EMPTY BOTTLES TO THE PHARMACY DEPARTMENT OF YOUR TREATING HOSPITAL Store at 2-8°C (do not freeze)	Keep out of reach of children For clinical trial use only / For oral use only ROCIT trial ANZCTRN: 12617000783325 PROBIOTIC Lactobacillus plantarum 299V 20 billion CFU or PLACEBO capsule (60 capsules) Take ONE capsule each day with a glass of water until all capsules have been taken. Bottle Number: XXXX Subject ID: XXXX Subject Name: _____ UMRN: _____ Date Dispensed: _____ Batch Number: Expiry:



ROCIT Protocol Supplementary Appendix

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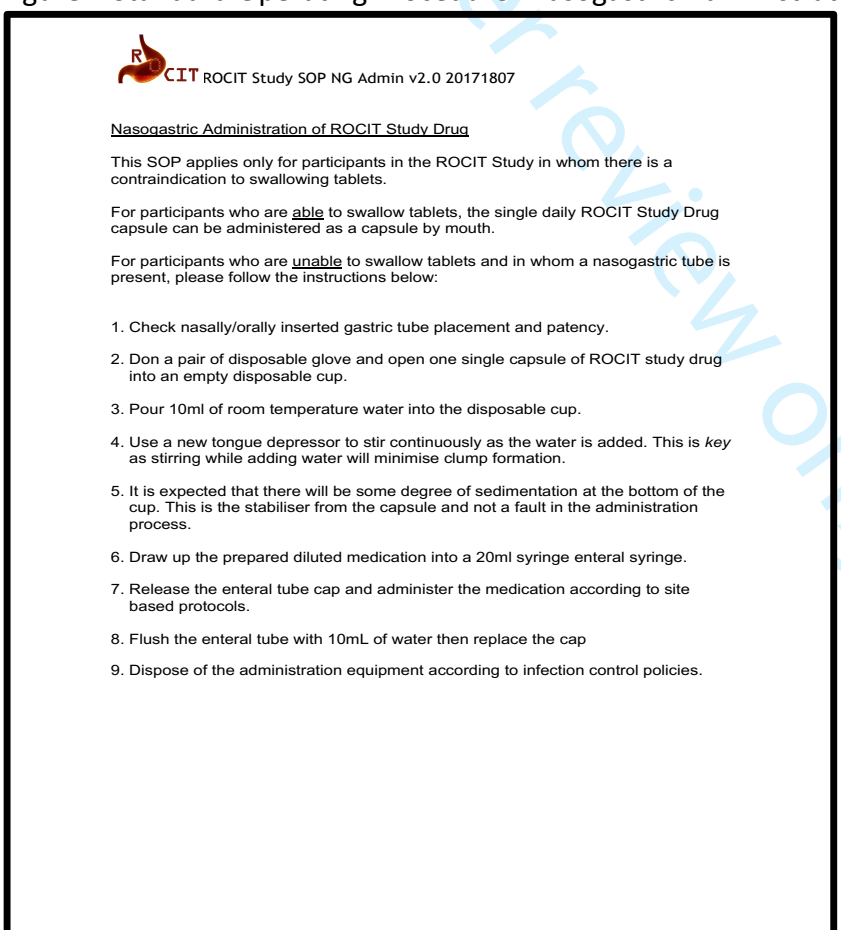
Figure 1 Standard Operating Procedure- Nasogastric Administration



ROCIT Study SOP NG Admin v2.0 20171807

Nasogastric Administration of ROCIT Study Drug

This SOP applies only for participants in the ROCIT Study in whom there is a contraindication to swallowing tablets.

For participants who are able to swallow tablets, the single daily ROCIT Study Drug capsule can be administered as a capsule by mouth.

For participants who are unable to swallow tablets and in whom a nasogastric tube is present, please follow the instructions below:

1. Check nasally/orally inserted gastric tube placement and patency.
2. Don a pair of disposable glove and open one single capsule of ROCIT study drug into an empty disposable cup.
3. Pour 10ml of room temperature water into the disposable cup.
4. Use a new tongue depressor to stir continuously as the water is added. This is *key* as stirring while adding water will minimise clump formation.
5. It is expected that there will be some degree of sedimentation at the bottom of the cup. This is the stabiliser from the capsule and not a fault in the administration process.
6. Draw up the prepared diluted medication into a 20ml syringe enteral syringe.
7. Release the enteral tube cap and administer the medication according to site based protocols.
8. Flush the enteral tube with 10mL of water then replace the cap
9. Dispose of the administration equipment according to infection control policies.

Table 1 Summary (abbreviated) of nosocomial infection diagnostic criteria*

Nosocomial infection	Criteria
Surgical site infection	Superficial incisional: occurs within 30 days after the operation and the infection involves only skin or subcutaneous tissue of the incision and at least one of the following: 1. Purulent drainage 2. Organisms isolated 3. At least one of pain, tenderness, swelling, redness, heat and superficial incision is deliberately opened by surgeon 4. Diagnosis of superficial incisional by surgeon Deep incisional: occurs within 30 days after the operation if no implant or within 1 year if implant and related to the operation and infection involves the deep tissues and at least one of the following: 1. Purulent drainage from the deep incision, but not from the organ of the surgical site 2. Deep incision spontaneously dehisces or is deliberately opened by the surgeon 3. Abscess or other evidence of infection involving the deep incision 4. Diagnosis of deep surgical space infection by the surgeon Organ/space: occurs within 30 days or within one year if implant is in place and related to the operation and infection involves any part of the anatomy other than the incision and: 1. Purulent drainage from a drain into the organ space 2. Organisms isolated aseptically from the organ/space 3. Abscess from the organ/space 4. Diagnosis of an organ/space infection by the surgeon The CDC criteria for burns infection will be included as a surgical site infection.
Blood stream infection	Clinical criteria are only required if the organism identified is a common skin contaminant Clinical criteria (at least one of): 1. Fever 2. WBC>10,000 or <3,000 per cubic millimetre 3. Hypotension (SBP<90) or >25% drop in SBP
C. difficile-associated diarrhoea	Clinical evidence of at least one of the following: 1. Pseudomembranes identified at lower gastrointestinal endoscopy

	2. Pathological confirmation of pseudomembranous colitis 3. C difficile toxin detected in the stool
Urinary tract infection	Clinical criteria of a and b must be satisfied within a two-day period a) Clinical criteria (at least one of): i. fever >38.5 C ii. WBC>10,000 or <3,000 per cubic millimetre iii. Urgency iv. Dysuria v. Suprapubic tenderness b) Bacterial confirmation vi. $>10^5$ organisms per ml of urine
Pneumonia	Criteria a-c must be satisfied within a 48-hour period a) Radiologic criteria i. New infiltrate that persists for at least 24 hours b) Clinical criteria (one of) i. Temperature >38.5 or <35 C ii. WBC>10,000 or <3,000 per cubic millimetre c) Bacterial confirmation (at least one of) i. BAL $>10^3$ CFU/ml ii. Histopathological examination of lung tissue iii. Positive blood culture for bacterial pathogen identified in sputum iv. Positive pleural culture with same organism in sputum v. Positive gram stain vi. Heavy or moderate growth of one type of pathogenic bacteria on semi-quantitative sputum culture Ventilator-associated pneumonia occurs where a patient is on mechanical ventilation for > two calendar days on the date of the event, with day of ventilator placement being day one, and the ventilator was in place on the date of the event or the day before.

WBC white blood cell, SBP systolic blood pressure, BAL bronchoalveolar lavage, CFU colony forming units. *For full criteria and wording see CDC. National Healthcare Safety Network (NHSN) Patient Safety Component Manual. Chapter 17: CDC/NHSN Surveillance Definitions for Specific Types of Infections. 2019. Where any difference occurs between this summary and the CDC definitions, the CDC definitions will take priority.

Figure 2. Study drug diary

Figure 2. Daily Medication Diary

ROCIT Study daily medication diary

1□	2□	3□	4□	5□	6□
7□	8□	9□	10□	11□	12□
13□	14□	15□	16□	17□	18□
19□	20□	21□	22□	23□	24□
25□	26□	27□	28□	29□	30□

Date of Hospital Discharge: Participant #:

Please record daily probiotics taken by ticking the box and make any comments on the day in the space provided.
Please keep and return the study bottles.
Any questions please contact the Dr Ed Litton or the Research Coordinators **via FSH switch board on 615 22222**

ROCIT Study daily medication diary

31□	32□	33□	34□	35□	36□
37□	38□	39□	40□	41□	42□
43□	44□	45□	46□	47□	48□
49□	50□	51□	52□	53□	54□
55□	56□	57□	58□	59□	60□

Please record daily probiotics taken by ticking the box and make any comments on the day in the space provided.
Please keep and return the study bottle.
Any questions please contact the Dr Ed Litton or the Research Coordinators via FSH switch board on 615 2222

Study Sites

1. Fiona Stanley Hospital, Robin Warren Drive, Perth, Western Australia
2. St John of God Hospital Subiaco, Salvado Road, Perth, Western Australia
3. St John of God Hospital Murdoch, Murdoch Drive, Perth, Western Australia
4. Sir Charles Gairdner Hospital, Nedlands, Perth, Western Australia
5. Royal Perth Hospital, Wellington Street, Perth, Western Australia

Collection and storage of biological specimens

Blood (plasma) and faeces will be collected from all study participants at four time points. These time points are:

1. Study enrolment (within 48h of admission to ICU or HDA)
2. 48h post enrolment or immediately prior to ICU discharge, whichever occurs first
3. 7 days post enrolment or immediately prior to hospital discharge, whichever occurs first
4. Day 60 post enrolment

Each blood sample will require a single 5ml serum tube. Feaces will be collected using a rectal swab by bedside clinical staff as is routine for patients admitted to the ICU and according to a standard operating procedure. For Day 60 samples in patients no longer remaining in hospital, a swab kit will be provided to the patient with simple standardised instructions on how to perform a rectal swab. This technique has been used in previous studies and found to be performed reliably. Samples will all be processed and stored within 2 hours from collection for biobanking, and in accordance with best practice recommendations for studies of the metabolome and microbiome.
Identification of the bacterial species of participant faecal samples will be performed using next generation sequencing. Measurement of the metabolome will be performed by liquid chromatography-mass spectrometry and will be targeted towards lipid and fatty acid metabolism (short chain fatty acids and Trimethylamine N-oxide).

The ROCIT Study

Patient Information and Consent Form

1. Introduction

You are invited to take part in this research project because you have been admitted to the Intensive Care Unit (ICU) or a high dependency area (HDA), and have been identified as potentially eligible to participate in the ROCIT Study.

This Patient Information and Consent Form tells you about the research project. It explains the procedures involved. Knowing what is involved will help you decide if you want to take part in the research project. Please read this information carefully. Ask questions about anything that you don't understand or want to know more about. Before deciding whether or not to take part, you might want to talk about it with a relative, friend or healthcare worker. This research project has been approved by the South Metropolitan Health Service Human Research Ethics Committee at Fiona Stanley Hospital.

Participation in this research project is voluntary. If you don't wish to take part, you don't have to. You will receive the best possible care whether you take part or not. If you decide you want to take part in the research project, you will be asked to sign the consent section. By signing it you are telling us that you:

- understand what you have read
- consent to take part in the research project
- consent to participate in the research processes that are described
- consent to the use of your personal and health information as described.

You will be given a copy of this Patient Information and Consent Form to keep.

2. What is the purpose of this research project?

The purpose of this research project is to find out if giving probiotic therapy (bacteria that may provide a health benefit) will reduce the risk of hospital acquired infections and lead to more rapid recovery for patients admitted to an ICU or HDA compared with current standard care that does not involve giving probiotics to patients in the ICU or HDA.

The probiotic used in this study (Plantarum 299v) is a bacteria found in the gastrointestinal tract of healthy people. Probiotics have several potentially beneficial properties including reducing the risk of growth of bacteria that may cause infection, protecting the lining of the gastrointestinal tract and reducing inflammation. Although there is some evidence that probiotics may be useful in critically ill patients, the data is currently insufficient to guide practice and probiotics are not used in the critical care areas of any of the participating institutions. However, the probiotic being studied is registered with the Therapeutic Goods Administration of Australia and is available for purchase 'over the counter' in Australian pharmacies. It is not an experimental product.

The ROCIT study will involve 220 patients admitted to critical care areas in several hospitals in Western Australia. The study has been funded by the State Health Research Advisory Council, Department of Health of Western Australia and has been designed by a collaborative group of clinicians and researchers.

3. What does participation in this research project involve?

The **ROCIT** trial team has assessed you as suitable to participate in this research project. If you agree to participate, you will be randomly assigned (like the toss of a coin) to receive either probiotic therapy in addition to standard care or placebo (sugar pill) in addition to standard care. For the purposes of this study and this Information Sheet, the term 'placebo' refers to the sugar pill. A placebo is an inactive and harmless 'treatment' given to the patients in the study who are randomised not to receive probiotics. Standard care is the care that you have, and will continue to receive, as a result of your current condition whether or not you agree to continue to take part in this study. You have a one in two chance of receiving probiotics or placebo in addition to standard care. Participants randomised to probiotics will receive a once daily administration of a single strain probiotic capsule containing 20 billion colony forming units of Lactobacillus plantarum strain 299v. Participants randomised to placebo will receive a once a day placebo capsule. All other treatment will remain at the discretion of the treating team.

The study is 'blinded'. This means that you, your relative, the researchers and the clinical staff caring for your relative will not know whether you are receiving probiotics or placebo. The blinding and the placebo are used to ensure that the results are a true reflection of the effect of the probiotic therapy. For this study, 'blinding' will be conducted by providing the study drug in identical packaging and ensuring that the probiotic and placebo capsules also look identical. The researchers will tell your doctors if they need to know which treatment has been received. The techniques of blinding and use of placebo are essential for the conduct of high quality clinical trials.

Treatment

If you agree to participate you will receive one capsule daily of either probiotic therapy or placebo whilst in the hospital and after discharge from hospital, for a maximum total study period of 60 days. At the time of hospital discharge, you will be provided with study medication and, where feasible, sent reminders by text message and phone call till the end of the total study period of 60 days. You will be asked to record your use of study medication daily. You will be visited at day 60 by study staff to collect your used bottles of study medication appointments or alternatively the participant can return it to the pharmacy of the hospital in which they were treated. .. At day 60 you will be asked to complete a short (10 minutes or less) questionnaire on how you are feeling and functioning. If you prefer to be followed up at day 60 in hospital, the study team will attempt to arrange this when you are also scheduled to return for other medical The study-related treatments and procedures are as follows:

- You will be assigned to receive either probiotics or placebo delivered as a capsule once daily from enrolment in the study to a maximum of 60 days.
- Whilst in the ICU, hospital ward or after discharge from hospital, a total of four rectal swabs are required (days 1,3,7 & 60). Rectal swabs are routinely taken

on admission to ICU to screen for infection. The additional swabs for this study will be used to determine the bacteria in your gastrointestinal tract. Measuring this at four time points will give the study investigators an understanding of changes over time as recovery takes place, and also the potential benefit of the probiotics.

- Whilst in the ICU and hospital ward a total of three blood samples will be taken in addition to the standard blood tests required by the medical team caring for you. Each will be a single blood tube of approximately 5 ml (or one table spoon) drawn, where available, from an existing intravascular device. The blood tests will be used to measure changes in how metabolism is occurring over time and the influence of the probiotics on this process. An additional fourth blood test at day 60 will be requested from participants who are returning to the hospital for clinical need. Failure to collect this blood sample does not preclude participation in the ROCIT study.
- During the study period, you will be continuously monitored whilst in hospital to enable the detection of any problems or complications. You will also be provided with contact details in the case of any concerns after discharge from hospital.
- Data will continue to be collected from the hospital medical notes and tests ordered by the clinical team treating you until the end of the study period. If required, we may also, with your permission, contact your GP to request clarification of clinical events related to the study.
- Samples will be stored at the hospital at which they are taken, then transferred via Harry Perkins FSH for analysis at:
  Cryptick Lab,
  Murdoch University
  90 South Street
  Murdoch WA 6150

4. What are the possible benefits?

We cannot guarantee or promise that you will receive any benefits from this research; however there may be possible benefits for patients admitted to the ICU or HDA in the future.

5. What are the possible risks?

Possible risks are from complications of study treatment. The study has been designed in a manner to ensure that the welfare of participants is protected at all times and that respect for patient autonomy is demonstrated through appropriate mechanisms of consent.

Probiotic therapy is available as over-the-counter medication in Australia and we do not expect adverse events related to the study treatment with probiotics. However, adverse events are possible. We will be screening for infection throughout the study period. Any suspected or confirmed infection related to probiotics will result in cessation of study medication. The probiotics being used in the study have been tested and known to be sensitive commonly used antibiotics in the event of suspected probiotic-associated infection. The placebo used in this study contains a standard inert material commonly used in placebo medication.

As occurs in routine clinical practice, all patients are monitored closely for development of any side effects from treatments which will be immediately treated by amending or stopping the treatment. There may be additional risks that the researchers do not expect or do not know about. You should tell a member of the research team immediately about any unusual symptoms that you get.

6. What if new information arises during this research project?
During the research project, new information about the risks and benefits of the project may become known to the researchers. If this occurs, you will be told about this new information and the researcher will discuss whether this new information affects you.

7. Are there alternatives to participation?
Participation in this research is not your only option. If you do not participate in this study, you will receive standard medical treatment. You can discuss this with your healthcare worker and any family members or close friends before deciding whether or not you should take part in this research project.

8. Do I have to take part in this research project?
Participation in any research project is voluntary. If you do not wish to take part, you do not have to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

Your decision whether you take part or not, or to take part and then withdraw, will not affect your routine treatment or your relationship with those treating you or your relationship with this Fiona Stanley Hospital.

9. What if I withdraw from this research project?
If you decide to withdraw from the project, please notify a member of the research team before you withdraw. This notice will allow that person or the research supervisor to inform you if there are any special requirements to withdrawing. The researchers would like to keep the personal and health information about you that have already been collected. This is to help them make sure that the results of the research can be measured properly.

10. Could this research project be stopped unexpectedly?
This research project may be stopped for a variety of reasons. These may include reasons such as unacceptable side effects, the treatment being shown not to be effective or the treatment being shown to work and not need further investigation.

11. How will I be informed of the results of this research project?
Once the project has been completed, a summary of the results will be available from the Chief Investigator Dr Ed Litton at Fiona Stanley Hospital on request.

12. What else do I need to know?

What will happen to information about me?
The information gathered about you by the investigator or obtained during the trial from blood tests or other procedures will be held by the investigator in strict confidence. Information from your medical records is essential to evaluate the results of this study. Information relevant to the research will be recorded on the

understanding that it will be treated confidentially. Your GP may also be contacted. Your trial records **without your name attached** will be made available to the study management committee and through publication in the peer-reviewed medical literature to government regulatory bodies in Australia and overseas. All the people who handle your information will adhere to traditional standards of confidentiality and will also comply with all relevant privacy legislation. In Australia this is the Privacy Act 1988. If the results of the trial are published in a medical journal, as is intended, no reader will be able to identify individual patients.

How can I access my information?

In accordance with relevant laws, you have the right to access the information collected and stored by the researchers about you. You also have the right to request that any information, with which you disagree, be corrected. Please contact one of the researchers named below if you would like to access your information.

Action if an adverse event arises during the trial

In the event that you suffer an adverse event or a medical accident during this study that arises from your participation in the study, you will be offered all full and necessary treatment by this Hospital. The South Metropolitan Health Service Human Research Ethics Committee has approved this study on the basis (amongst others) that the reported risk of such an event is either small or acceptable in terms of the risk you face as a result of your current illness or the benefit that is possible with the new treatment being tested. No provisions have been made in this trial to offer trial subjects who suffer an adverse reaction monetary compensation, but the absence of such a provision does not remove your rights to seek compensation under common law.

Whom should I contact if I need more information?

If you have any questions please contact:

Dr Edward Litton
Intensive Care Unit
Fiona Stanley Hospital
Tel: 08 6152 6547

Susan Pellicano
Project Coordinator
Fiona Stanley Hospital
Tel:08 6152 6546

If you have questions about your rights as a research Participant, you may contact:
the South Metropolitan Health Service Research Ethics and Governance Unit.
Tel: (08) 6151 1180
Email: SMHS.REG@health.wa.gov.au

Study - Patient Consent Form

Name of Patient _____ Date of Birth_____

1. I agree voluntarily to take part in Multicentre Study: ROCIT Study. This study has been funded by the State Health Research Advisory Council of W.A. I am 18 years of age or over.

2. I have been given a full explanation of the purpose of this study, of the procedure involved and of what will be expected of me. The doctor has explained the possible problems that might arise as a result of my participation in this study.

3. I agree to inform the supervising doctor of any unexpected or unusual symptoms I may experience as soon as possible.

4. I understand that I am entirely free to withdraw from the study at any time and that this withdrawal will not in any way affect my standard or conventional treatment or medical management.

5. I understand that the information in my medical records is essential to evaluate the results of this study. I agree to the release of this information to the research staff and the clinical staff on the understanding that it will be treated confidentially.

6. I agree to my General Practitioner (GP) being informed about my participation in this study and to my GP providing clarification of clinical events related to the study.

7. I understand that I will not be referred to by name in any report concerning this study. In turn, I cannot restrict in any way the use of the results that arise from this study.

8. I have been given and read a copy of this Consent Form and Information Sheet.

Signature of Patient..... Signature of Doctor.....

Name of Patient: ..... Name of Doctor.....

Date: ..... Date: .....

Time: ..... Time: .....

Table 2. Adverse Events

System	Clinical Features	Time of onset	Duration	Resolution

SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

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

Section/item	Item No	Description
Administrative information		
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym – page 1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry – page 3&5
	2b	All items from the World Health Organization Trial Registration Data Set – page 3
Protocol version	3	Date and version identifier – page 3
Funding	4	Sources and types of financial, material, and other support – page 18
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors – page 1,2,15-18
	5b	Name and contact information for the trial sponsor – page 2
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities – page 17-18
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) – page 11 for Data Monitoring Committee
Introduction		
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention – page 4
	6b	Explanation for choice of comparators -page 4-5
Objectives	7	Specific objectives or hypotheses – page 5

Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) – page 5
Methods: Participants, interventions, and outcomes		
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained – page 6 & Supplementary appendix
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) – page 6 and Table 1
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered page 7-8
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) – page 8
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) – page 7&9
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial – page 8
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended – page 8&9
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) – page 7
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations – page 9 &10
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size – page 6
Methods: Assignment of interventions (for controlled trials)		
Allocation:		

Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions – page 6
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned – page 6
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions – page 6
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how – page 6
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial – page 6

Methods: Data collection, management, and analysis

Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol – page 9
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols – page 9
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol – page 9
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol – page 9&10
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses) – page 10
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) – page 10

Methods: Monitoring

Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed – page 11
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial – page 10
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct – page 10&11
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor – page 10

Ethics and dissemination

Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval – page 12
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) – page 12
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) – page 6
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable – all study procedures were described in the primary consent form.
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial – page 9&10
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site – page 15-18
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators – page 12
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation – n/a

Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions – page 12
	31b	Authorship eligibility guidelines and any intended use of professional writers – page 15-18
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code – page 12
Appendices		
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates – page 12 & Supplementary appendix
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable – page 9 & Supplementary appendix

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