
# Microbiology Appendix

## (Operational Document)

# **R**and**O**mised **A**rthroplasty infection worl**D**wide **M**ultidomain **A**daptive **P**latform (ROADMAP) Trial

**Clinical Trial Registration Trial ID**: NCT06771050

Microbiology Appendix Version 2.0, dated 19 December 2025

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# 1. MICROBIOLOGY APPENDIX VERSION

The version of the Microbiology Appendix is in this document's header and on the cover page.

## *1.1 Document History*

| Version Number | Date | Summary of Changes |
|---|---|---|
| 1.0 | 26 June 2025 | • Initial version approved by the Microbiology Working Group (MWG) |
| 2.0 | 19 December 2025 | • Minor formatting and wording changes to improve clarity.<br>• Expansion of the contact list and their relevant details.<br>• Inclusion of the laboratory procedures for the collection, transport and storage of isolates at both a regional and global level.<br>• Inclusion of a statements on ownership and sharing of isolates |
| | | |

### *1.2. Quick Reference Summary*

| | |
|---|---|
| **Site:** | A participating clinical or hospital-based location enrolled in the ROADMAP trial that recruits patients and collects clinical specimens. |
| **Regional Repository:** | A Regional Repository is a designated facility responsible for receiving, processing, and securely storing biological specimens within a defined geographic region. It acts as an intermediate hub for temporary storage before samples are transferred to the Global Repository (Hunter Medical Research Institute (HMRI), Newcastle, New South Wales (NSW), Australia). |
| **Global Repository:** | The Global Repository is the central, long-term storage facility that receives specimens and data from all participating Regional Repositories worldwide. It provides unified specimen management, long-term preservation under standardised conditions, and coordinated access for approved sub-studies. The Global Repository serves as the definitive reference collection for the ROADMAP trial, ensuring harmonised data integration, traceability, and global oversight of specimen utilisation. |
| **Specimen types:** | Bacterial isolates grown from synovial fluid (intraoperative or percutaneous), intraoperative tissue, sonicate fluid, intraoperative sterile swabs or blood cultures. See **Section 8.3** for more details including preference hierarchy.<br><br>**Note:** isolates grown from non-sterile swabs and/or other non-sterile site specimens should not be stored. |
| **Representative isolate:** | One (1) isolate per patient episode provided in duplicate, in consultation with the site Principal Investigator (or delegate) – see **Section 8.3** for criteria for choosing the representative isolate. |
| **Criteria for isolate selection:** | Microbiological criteria for a 'confirmed' (or suspected) prosthetic joint infection as defined by the European Bone and Joint Infection Society criteria (See **Figure 2**).<br>**Note:** while the EBJIS criteria for microbiologically confirmed PJI are encouraged, we recognise that clinical judgment may diverge. |
| **Recommended Storage:** | −80 °C in sterile glycerol (≥ 20%) in labelled cryovials (or equivalent method) in duplicate (see **Section 8.6**).<br><br>While sites may implement their own internal workflows and systems it is expected that specimens intended for transfer to the Global Repository are prepared, packaged, and documented in alignment with the procedures described in this document, by the Regional Repositories. |

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| **Transport:** | **Regional:** Each ROADMAP region (Australia, New Zealand, Canada, Europe, and the United Kingdom) will host and manage its own Regional isolate Repository, coordinating and funding shipments from site laboratories every 6 – 12 months via approved courier.<br><br>**International:** Every 2 – 4 years, all regional isolates will be transferred to the Global ROADMAP Repository at the HMRI (Australia), where they will be stored with their associated metadata.<br><br>See **Sections 10, 11 and 12** for more details |
| **Sample Labelling:** | Each isolate vial must be labelled with the ROADMAP participant ID, date of sample collection (DD/MM/YYYY), organism code, laboratory isolate number (if applicable), and replicate identifier. Two duplicate vials must be prepared for every isolate and labelled as Replicate A and Replicate B. See **Section 8.7.1**. |
| **Documentation:** | Email electronic copy of Specimen Transport Log to the Repository Coordinator (both Regional and Global) and the Trial Management Group (TMG) before shipment. A hard copy must also accompany the shipment. See **Section 17.** |
| **Renumeration and responsibility:** | Each ROADMAP region is responsible for managing and funding its Regional Isolate Repository, including local storage and routine collection and shipment of isolates from participating laboratories. In Australia, ROADMAP reimburses sites for storage of one isolate in duplicate per participant episode, while additional isolates are stored at the site's own cost; for New Zealand sites, isolate costs are included in the participant reimbursement, and for other international sites, storage is managed locally with the ROADMAP central team covering international shipping only. See **Sections 13 and 15.** |
| **Restrictions:** | Avoid weekend arrivals; ensure international air transport association packing instruction 650 compliance (see **Section 11** for details). |
| **Queries:** | Contact the Repository Coordinator or TMG for general inquiries. For region-specific advice, please contact the appropriate Regional Team, and for global matters, contact the Global Team. |

## 2. MICROBIOLOGY WORKING GROUP GOVERNANCE

The ROADMAP MWG and the ROADMAP Global Trial Steering Committee (GTSC) will serve as key forums for information sharing and discussion. Each region is expected to have representation within these groups to ensure broad oversight and input.

### *2.1 Trial Governance and Working Group Members*

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|---|---|
| **Co-ordinating Principal Investigators:** | **Professor Joshua Davis**<br>**Email:** joshua.davis@health.nsw.gov.au<br>**Professor Laurens Manning**<br>**Email:** laurens.manning@uwa.edu.au |
| **Project Manager:** | **Dr Marline Squance** – ROADMAP Global Clinical Trial Manager. Hunter Medical Research Institute, Level 4, West Wing Lot 1, Kookaburra Circuit, New Lambton Heights, Newcastle, New South Wales, Australia.<br>**Email:** marline.squance@newcastle.edu.au |
| **Working Group Chair:** | **Dr Stefano Giulieri**[1, 2] (Australia, Microbiology lead)<br>**Email:** stefano.giulieri@unimelb.edu.au |
| **Working Group Members**: | **Professor Joshua Davis**[3,4] (Australia)<br>**Professor Laurens Manning**[5,6] (Australia)<br>**Dr Bernadette Young**[7,8] (United Kingdom)<br>**Dr Marisa Azad**[9,10] (Canada)<br>**Dr Marline Squance**[3,4] (Australia)<br>**Dr Paul Ingram**[11,12,13] (Australia)<br>**Dr Liz Sheridan**[14,15,] (United Kingdom)<br>**Dr Susan Harch**[16,17] (Australia)<br>**Dr Susan Morpeth** [18,19,] (New Zealand)<br>**Dr Syeda Naqvi**[4,20] (Australia)<br>**Dr Stéphane Corvec**[21] (France) |
| **Working Group Member Affiliations:** | 1. Victorian Infectious Diseases Service, The Royal Melbourne Hospital, Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia.<br>2. Department of Microbiology and Immunology, The University of Melbourne at the Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia.<br>3. School of Medicine and Public Health, University of Newcastle, Newcastle, New South Wales, Australia. |

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|---|---|
| | 4. Infection Research Program, Hunter Medical Research Institute, New Lambton Heights, Newcastle, New South Wales, Australia.<br>5. School of Medicine, University of Western Australia, Perth, Western Australia, Australia.<br>6. Department of Infectious Diseases, Fiona Stanley Hospital, Murdoch, Perth, Western Australia, Australia.<br>7. Bone Infection Unit, Nuffield Orthopaedic Centre, Oxford University Hospitals, Oxford, OX3 7LD, United Kingdom.<br>8. Nuffield Department of Medicine, University of Oxford, Oxford, OX3 9DU, United Kingdom.<br>9. Division of Infectious Diseases, Department of Medicine, University of Ottawa at The Ottawa Hospital, Ottawa, ON, K1H 8L6, Canada.<br>10. Ottawa Hospital Research Institute, Ottawa, ON, K1H 8L6, Canada.<br>11. Department of Microbiology, PathWest Laboratory Medicine, Perth, Western Australia, Australia.<br>12. Department of Infectious Diseases, Royal Perth Hospital, Perth, Western Australia, Australia.<br>13. Department of Pathology and Laboratory Medicine, University of Western Australia, Perth, Western Australia, Australia.<br>14. Department of Life & Environmental Sciences, Talbot Campus Fern Barrow, Bournemouth University, Poole BH12 5BB, United Kingdom.<br>15. Department of Medical Microbiology, Poole Hospital, University Hospitals Dorset NHS Foundation Trust, Longfleet Road, Poole BH15 2JB, United Kingdom.<br>16. Department of Infectious Diseases, Royal Prince Alfred Hospital, Sydney, New South Wales, Australia.<br>17. Department of Microbiology, NSW Health Pathology Royal Prince Alfred Hospital, Sydney, Australia<br>18. Department of Infectious Diseases, Middlemore Hospital, Te Whatu Ora Counties Manukau, Auckland, New Zealand. |

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| | 19. Departments of Microbiology and Infectious Diseases, Middlemore Hospital, Te Whatu Ora Counties Manukau, Auckland, New Zealand.<br>20. Department of Infectious Diseases, John Hunter Hospital, Newcastle, New South Wales, Australia.<br>21. Bacteriology and Hygiene Department, Centre Hospitalier Universitaire de Nantes, Nantes, France. |
| **Global Isolate Repository Coordinator:** | **Dr Jacinta Martin**<br>Hunter Medical Research Institute, Level 4, West Wing Lot 1, Kookaburra Circuit, New Lambton Heights, Newcastle, New South Wales, Australia.<br>**Email:** isolate_repository@roadmaptrial.com |
| **Central Trial Management Office:** | **ROADMAP Trial Management Team (TMG)**<br>Hunter Medical Research Institute, Level 4, West Wing Lot 1, Kookaburra Circuit, New Lambton Heights, Newcastle New South Wales, Australia.<br>**Email:** tmg@roadmaptrial.com.au |

## *2.2 Microbiology Working Group Authorisation*

The Microbiology Working Group have read the appendix and authorise it as the official Microbiology Appendix for the ROADMAP trial.

Signed on behalf of the committee,

**Chair**

Date 19/12/2025

Dr Stefano Giulieri

# 3. DEFINITIONS AND ABBREVIATIONS

## *3.1 Definitions*

| Term | Explanation |
|---|---|
| **Microbiological criteria for a 'confirmed' prosthetic joint infection (PJI).**<br><br>**Definition from the European Bone and Joint Infection Society (EBJIS)**[1] | 1. The same microorganism has been identified in two (2) or more intraoperative fluid or tissue samples; OR<br>2. Any organism has been grown from sonicated fluid (from prosthesis or components) with >50 colony forming units (CFU)/ml (or >200 CFU/ml if centrifugation is used). |
| **Index isolate** | The most clinically important isolate during this episode of infection from one (1) or more samples from the infected prosthetic joint (joint fluid, tissue biopsy or sonication fluid), according to the hierarchy outlined in **Section 8.3**.<br><br>If no significant organisms are grown from joint samples, but a significant pathogen is grown in blood cultures, (e.g. because of revision after starting antibiotics), a blood culture isolate will be considered the causative organism. |
| **Representative isolate** | Frequently multiple specimens and sample types are culture positive. A single colony will be stored (typically from the isolate from which susceptibility testing is performed) to "represent" the most important pathogen in this patient's episode.<br><br>The representative isolate should be selected (in order of preference) from intraoperative tissue, synovial fluid (intraoperative then percutaneous), sonicate fluid, or if otherwise culture-negative, from blood cultures or intraoperative sterile swabs. Please note, isolates obtained from non-sterile swabs should not be stored. |
| **Repeat isolate** | An isolate of the same species collected from the infected prosthetic joint $\geq$ 30 days after the index isolate. |
| **Polymicrobial infections** | The simultaneous presence of multiple microorganisms contributing to an infection. |

### *3.2 Abbreviations*

| Abbreviation | Explanation |
|---|---|
| ARREST | Adjunctive Rifampicin for *Staphylococcus aureus* Bacteraemia Trial |
| CFU | Colony Forming Units |
| $CO_2$ | Carbon Dioxide |
| CRF | Case Report Form |
| EBJIS | European Bone and Joint Infection Society |
| EUCAST | European Committee on Antimicrobial Susceptibility Testing |
| EOI | Expression of Interest |
| GTSC | Global Trial Steering Committee |
| HREC | Human Research Ethics Committee |
| HMRI | Hunter Medical Research Institute |
| IATA | International Air Transport Association |
| ID | Identification / Identifier |
| MIC | Minimum Inhibitory Concentration |
| MTA | Material Transfer Agreements |
| MWG | Microbiology Working Group?? |
| NSW | New South Wales |
| PI | Principal Investigator |
| PJI | Prosthetic Joint Infections |
| REDCap | Research Electronic Data Capture |
| ROADMAP | RandOmised Arthroplasty infection worlDwide Multidomain Adaptive Platform |
| SOP | Standard Operating Procedure |
| TMG | Trial Management Group |
| UN | United Nations |
| UON | University of Newcastle |
| WG | Working Group |

# 4. ETHICAL CONSIDERATIONS FOR THE USE OF CLINICAL ISOLATES

Clinical isolates used within the ROADMAP Repository do not require separate Human Research Ethics Committee (HREC) approval. These materials are bacterial specimens that have been collected as part of standard diagnostic and clinical management procedures. Under the National Statement on Ethical Conduct in Human Research, ethics approval is required only when research involves identifiable or re-

identifiable human participants, human tissue, or personal health information. Because the isolates included here:

- contain **no human tissue** (i.e. are purely microbial samples),
- are de-identified before transfer to the study team,
- were generated during routine clinical workflows rather than for research-specific purposes, and
- are incapable of being traced to a patient or used to derive personal information,

their use falls **outside the scope of human research** as defined by the National Statement.

For these reasons, the collection, storage, and analysis of de-identified bacterial isolates for this study can proceed without HREC review. This approach is consistent with standard practice across Australian clinical research involving microbiological repositories and is supported by guidance provided by institutional ethics offices and jurisdictional advisory bodies.

**Note:** International regional repositories must confirm how this position aligns with their own jurisdictional, institutional, or national regulatory requirements and act accordingly.

## 5. PURPOSE AND SCOPE:

The **R**andomised **A**rthroplasty infection worl**D**wide **M**ultidomain **A**daptive **P**latform (ROADMAP) Trial will investigate treatments for prosthetic joint infections (PJI) in multiple management domains including *Antibiotic Choice, Antibiotic Duration* and *Surgical Management*. For further information on the trial please consult the core protocol and domain specific appendices, all of which are available on the ROADMAP website (https://www.roadmaptrial.com).

This document outlines:

i) the suggested microbiological workflow at the clinical microbiology / pathology laboratories servicing trial sites,
ii) the rationale, structure and details of the ROADMAP isolate repository (both Global and Regional); and
iii) information about the transport, subsequent use, sharing and ownership of the isolates in the repository.

In contrast to other ROADMAP protocol documents, this Microbiology Appendix is an **operational document** which does not require ethics or governance approval.

# 6. APPLICABILITY AND CONTEXT:

This document is the global Microbiology Appendix. Its content is consistent with Australian practices for specimen handling, transport, and storage. ROADMAP recognises that **variations may exist between countries and regions** due to differing regulatory frameworks, biosafety standards, and ethical requirements. While it is preferred all regions follow the workflow in this appendix, a region can add a regional appendix to this appendix to outline region-specific variances. To do so please forward the documentation to the Global Isolate Repository Coordinator for review.

While **Regional Repositories** may implement their own internal workflows, systems, and local governance procedures, it is **expected that specimens intended for transfer to the Global Repository** are prepared, packaged, and documented in alignment with the procedures described in this document. Accordingly, this document serves as a **reference framework that can be adapted to local contexts while promoting standardisation** to ensure specimen integrity, traceability, and compatibility across the international ROADMAP network.

To support international consistency, the following definitions apply:

**Site:** A participating **clinical or hospital-based location** enrolled in the ROADMAP trial that recruits patients and collects clinical specimens. Specimens collected at the hospital site are processed and stored short-term at the associated **clinical microbiology or pathology laboratory** servicing that site.

**Regional Repository:** A **designated facility responsible for receiving, processing, and securely storing biological specimens from study sites within a defined geographic region** (e.g., within a country or group of neighbouring countries). It acts as an intermediate hub for quality control, tracking, and temporary storage before samples are transferred to the Global Repository. Regional Repositories ensure compliance with local biosafety, transport, and ethical regulations while maintaining consistent data linkage and sample integrity across sites.

**Global Repository:** The **central, long-term storage facility that receives specimens and data from all participating regional repositories worldwide**. It provides unified specimen management, long-term preservation under standardised conditions, and coordinated access for approved sub-studies. The Global Repository serves as the definitive reference collection for the project, ensuring harmonised data integration, traceability, and global oversight of specimen utilisation.

## *6.1 Repository Flow:*

Individual **trial sites** collect patient specimens, which are processed and held short-term within their associated **clinical microbiology / pathology laboratories**. Eligible isolates will then be forwarded—

together with accompanying documentation—to their designated **Regional Repository** for medium-term storage. Each **Regional Repository** will collate submissions from its participating sites and prepare consolidated shipments to the **Global Repository** in accordance with the procedures and specifications outlined in this document for long-term preservation. This tiered structure ensures a clear chain of custody, enables regional oversight and quality assurance, and supports consistent specimen management across all participating countries (See **Figure 1**).


**Figure 1: Overview of isolate transfer within the international ROADMAP network.** This map illustrates the specimen and isolate transfer pathway between trial sites, Regional Repositories, and the Global Repository under the ROADMAP framework. Participating hospital or clinical sites, where patients are recruited and specimens are collected, are represented by teal (Australia), yellow (New Zealand), mustard (France) and red (Canada) icons. Regional Repositories, shown in dark purple (Australia), maroon (New Zealand), orange (France) and light purple (Canada), are designated facilities responsible for collating, and securely storing isolates received from sites within their geographic region. The Global Repository at the Hunter Medical Research Institute (HMRI), represented by the dark purple icon, is the central long-term storage facility that receives isolates from all Regional Repositories. Connecting lines indicate the flow of isolates and associated documentation from sites to Regional Repositories and then to the Global Repository. **Note**: In Australia, the Global Repository also serves as the Australian Regional Repository. This map is for illustrative purposes only and some site locations are fictional.

## 7. GENERAL GUIDANCE ON LABORATORY WORKFLOW

**ROADMAP is a pragmatic trial**; accordingly, sites and their laboratories are expected to follow their usual diagnostic workflows for the detection and identification of microorganisms in clinical specimens

from patients with suspected or proven PJI, provided these are consistent with generally accepted guidelines[2]. These include sending at least five (5) specimens of tissue, fluid, or bone from a suspected infected joint that has been opened intra-operatively, using a separate set of sterile instruments for each sample, and ensuring that the receiving laboratory cultures them under both aerobic and anaerobic conditions. Laboratories may, at their discretion, also utilise adjunctive methods such as sonication of retrieved implants, nucleic acid amplification tests, or extended duration incubation.

The **ROADMAP protocols (including this appendix) do not dictate microbiological methods for this routine laboratory work** – only for the subsequent selection and shipping of those isolates for the Global ROADMAP isolate repository.

# 8. CORE ISOLATE REPOSITORY

## *8.1 Rationale*

The rationale of the ROADMAP isolate repository is to:

- Enable retrospective phenotypic and genotypic analysis of rifampicin susceptibility, thereby supplementing clinical data obtained by the *Antibiotic Choice Domain,*
- Allow microbiological distinction between PJI reinfection and relapse,
- Provide the basis of sub-studies to investigate bacterial factors (e.g. bacterial diversity in clinical samples, antibiotic resistance and / or virulence) which may impact PJI treatment outcomes,
- Form a valuable resource for other future *in-vitro* research related to PJI (e.g. use in laboratory models of biofilm or PJI; susceptibility testing against novel antibiotics),
- Facilitate quality control studies.

## *8.2 Location*

Each participating ROADMAP region (currently Australia, New Zealand, Canada, Europe and the United Kingdom) will host a Regional isolate Repository. The regional management group and regional trial steering committees will co-ordinate their Regional Repositories and will retain ownership of it, including responsibility for arranging and funding reimbursement for isolate collection (see **Section 13** for more details on renumeration) and subsequent shipment of isolates from participating sites / pathology laboratories to the Regional Repository at intervals of approximately 6 - 12 months.

The Global ROADMAP isolate Repository will be housed at the HMRI in Newcastle, Australia. At intervals of 2 - 4 years, a complete copy of all isolates from each Regional Repository will be transferred to the HMRI, where it will be stored together with its metadata. **The ROADMAP central team will cover national/international shipping costs from the Regional Repository to the Global Repository.**

## *8.3 Sample Collection and Selection of the Representative Isolate*

All sites participating globally will be asked to collect and store a representative bacterial isolate for each confirmed culture-positive PJI case (see definition above and **Figure 2**). Only one (1) representative index isolate is required per platform participant.

Once the microbiological work-up is completed, the clinical site team should be notified of the microorganism(s) identified to guide selection of the key (representative) pathogen for that episode. The clinical team / investigator (or delegate) will then liaise with the laboratory to confirm which strain(s) cultured from the infected site are deemed clinically relevant and request that an isolate be prepared for storage in duplicate. This approach acknowledges the frequently heterogeneous nature of specimen type, number of positive samples, pathogen growth characteristics, and weighted microbiological significance.

Selection of the representative ("key") isolate must be undertaken in a stepwise manner, considering the following three factors in the order listed below.

**Note:** this stepwise approach applies when more than one organism is isolated from multiple specimen sources and/or at multiple collection times during the same episode.

1. **Organism type**
2. **Specimen type (source/location)**
3. **Collection time (earliest isolate)**

Sites should proceed through each step sequentially to identify the single isolate most appropriate for storage for that episode.

### 8.3.1 Step 1 – Organism type

**Monomicrobial infections:** For monomicrobial infections, the representative isolate should be the organism identified as clinically relevant for that episode.

**Polymicrobial infections:** For polymicrobial infections, the representative isolate—ideally the organism considered most likely to represent the primary causative pathogen, should be selected according to the following recommended hierarchy:

1. *Staphylococcus aureus*
2. *Streptococcus spp*
3. Coagulase negative staphylococci
4. Other gram-positive pathogens (including Enterococci, Cutibacterium, etc)
5. Gram negative pathogens and
6. Other

While the EBJIS criteria for microbiologically confirmed PJI are encouraged (see definitions above and **Figure 2**), clinical judgement may diverge. For example, a single positive sample for *Staphylococcus aureus*

may be considered clinically relevant, whereas multiple isolates of coagulase-negative staphylococci may be regarded as contaminants.

#### 8.3.2 Step 2 – Specimen type

Once the priority organism has been identified (Step 1), the representative isolate should be selected based on **specimen type (source/location)**.

The preferred source for the representative isolate, in order, is

1. intraoperative tissue,
2. intraoperative synovial fluid,
3. percutaneous synovial fluid,
4. sonicate fluid,
5. blood cultures or
6. intraoperative sterile swabs

**Note:** Isolates obtained by swabs from non-sterile sites should **not** be stored.

#### 8.3.3 Step 3 – Collection time

If the selected organism has been isolated more than once from the same specimen type, the first isolate collected should be selected.

### *8.4 Communication*

Each site Principal Investigator (PI) or delegate should discuss with their relevant pathology provider / laboratory, which PJI isolates qualify for storage and guide the choice of the priority organism for each specimen using the aforementioned guiding framework. The site PI (or delegate) is also responsible for notifying the laboratory when a patient is enrolled in the study, and for providing their study identifier for labelling (more details in **Section 8.7.1**). The identifier will include a unique three-letter site code (e.g. JHH for John Hunter Hospital) and the sequential enrolment number (e.g. JHH-00027). **Patient identifiers such as name or date of birth must not appear on stored isolates.**

Sites must **notify their clinical microbiology or pathology laboratory in real time** when a participant is enrolled in the ROADMAP trial, so the laboratory is aware that any subsequent isolates may require selection and storage. Once cultures have been processed and results are available, the site team will be informed by their laboratory. At that point, **sites must coordinate isolate selection and advise the laboratory of their chosen representative organism within the laboratory's specified allowable time-out period using the above guidelines.** These time limits vary by laboratory; however, in most cases notification of isolate selection must occur within one to two weeks of culture availability (unless otherwise stated).

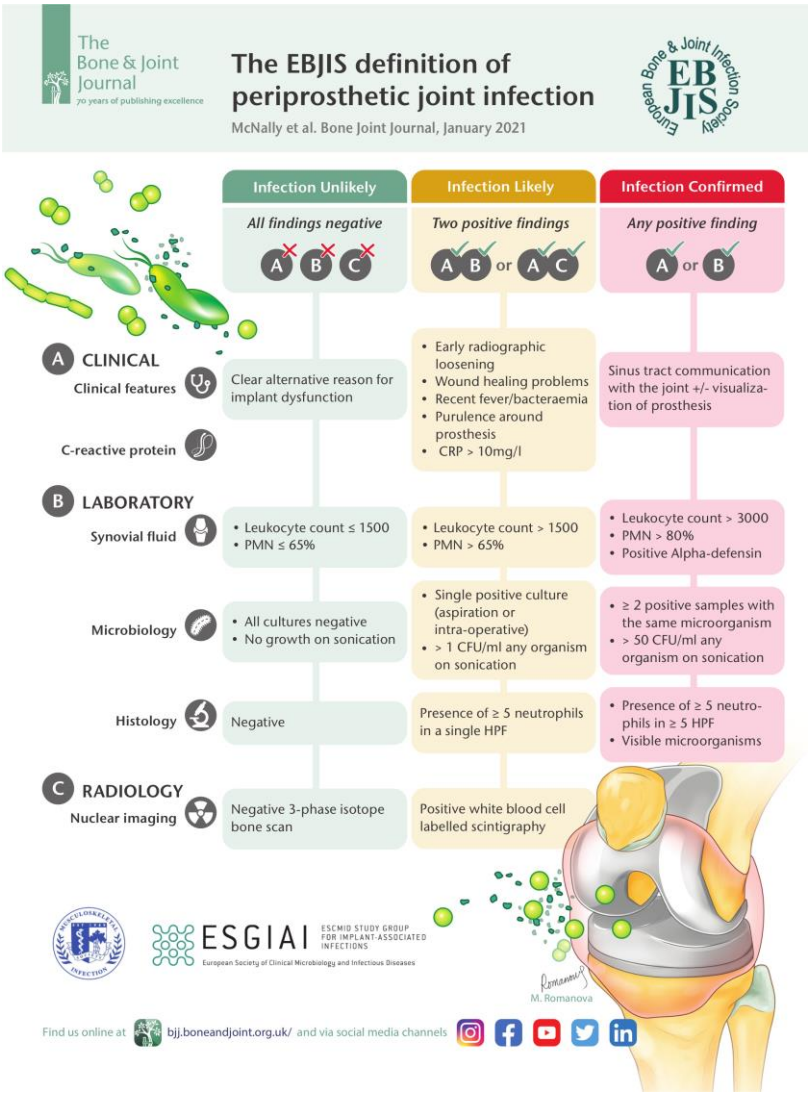


**Figure 2: The EBJIS definition of prosthetic joint infection (PJI), outlining criteria across clinical, laboratory, and radiological domains.** Infection is classified as unlikely if all findings are negative, likely if two findings are positive, and confirmed if any single major criterion is met[1].

**Figure 3** (below) provides a framework for determining which isolate(s) to store. In the event of uncertainty, ROADMAP encourages PIs to make their 'best judgment' and acknowledges the trial is pragmatically designed.

## *8.5 Suggested Laboratory Workflow*

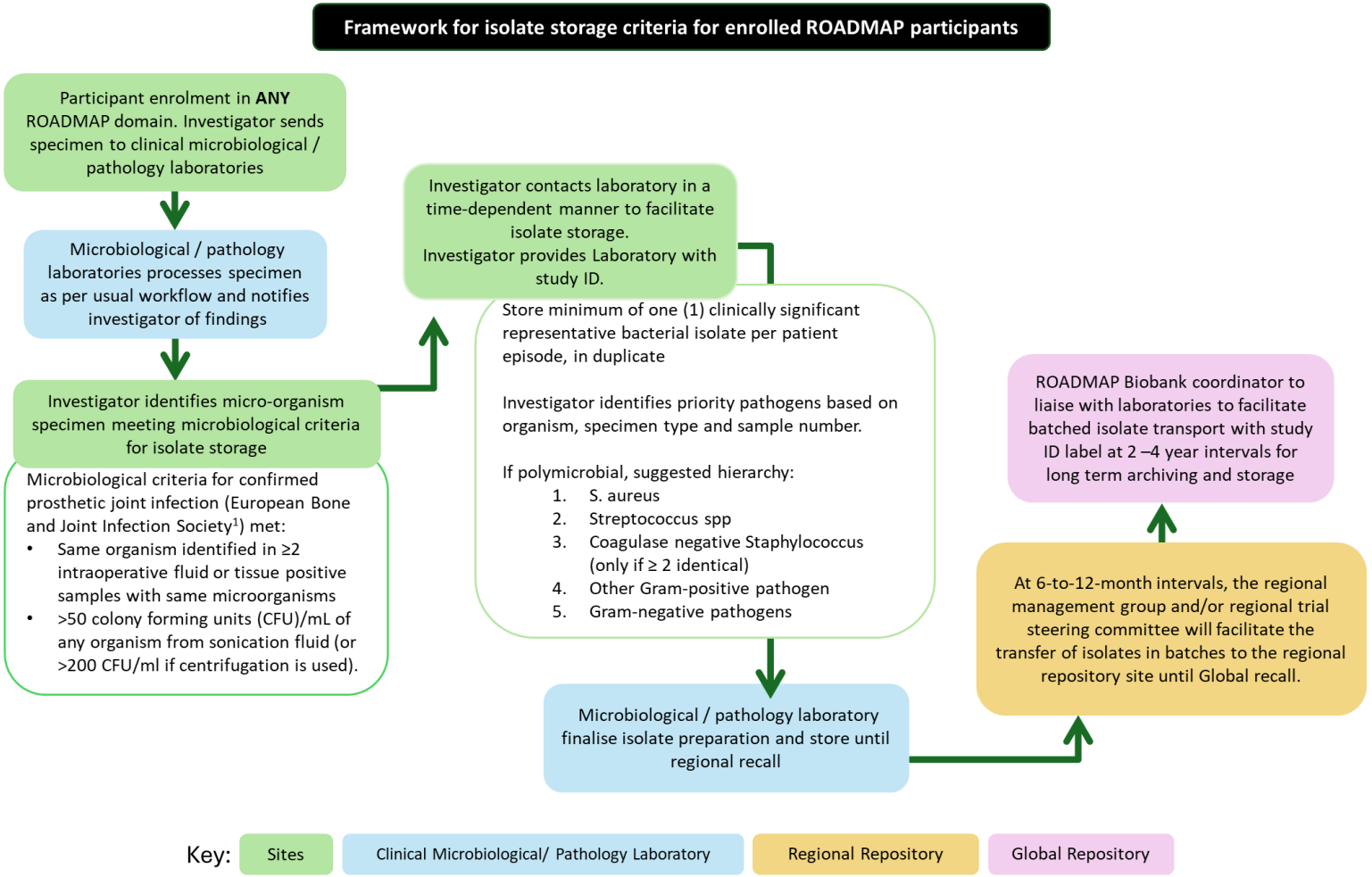


**Figure 3.** Isolate Storage Laboratory Workflow.

## *8.6 Sample Storage*

All PJI isolates must be frozen and stored in accordance with standard laboratory practice and labelled with a study-specific label (see example in **Section 8.7.1**). The label must include the ROADMAP study identifier, the date of isolate collection, and—where multiple or repeat isolates (See **Section 9**) are collected for a participant—the isolate number (e.g. 1, 2, 3).

ROADMAP recommends that a **pure subculture of the selected representative isolate be stored, in duplicate, at −80 °C in ≥20% sterile glycerol, using two (2) 2.0 mL screw-cap cryopreservation/freezing vials** (or an equivalent method, including cryobank ceramic bead systems where available).

As previously indicated, ROADMAP recognises that participating laboratories may operate under different local storage workflows. **Where laboratories are unable to prepare frozen glycerol stocks and can provide isolates only on swabs or agar slopes, the Regional Repository will be responsible for processing these materials and preparing a pure subculture, stored**, in duplicate, at −80 °C in ≥20% sterile glycerol in sterile 2.0 mL screw-cap cryopreservation/freezing vials (or an equivalent method), labelled as specified above. **Isolates submitted on swabs or slopes cannot be accepted directly by the Global Repository.**

Sites that already routinely store PJI isolates are asked to prepare three (3) copies of each selected isolate: one (1) to be retained on site for routine clinical or research purposes, and two (2) additional copies reserved for the ROADMAP trial.

## *8.7 Labelling*

The isolates need to be labelled for storage and transport with the details indicated below on the label. An example is provided below.

### 8.7.1 Required labelling elements

Each vial must include the following five components:

ROADMAP ID | COLLECTION DATE | ORGANISM CODE | LAB # | REPLICATE ID

1. Participant Study Number (e.g., AUJHH-00027) [ ]
2. Date of Sample Collection (DD/MM/YYYY) [ ]
3. Organism Code [ ]
    - Short alphanumeric code (e.g., ECO for E. coli, SAU for S. aureus, CNS for coagulase-negative staphylococci, CAN for Candida spp.)

**Note 1:** A standardised code list is provided in **Section 17, Appendix 17.1**.

**Note 2:** Sites may use their laboratory's existing organism code list, but a copy of the code must be provided to the ROADMAP Biobank Co-ordinator for reference.

4. Laboratory Isolate Number (as issued by the microbiology laboratory (if required))—from samples that have polymicrobial growth. [ ]
    - Where applicable, this may include suffixes (e.g. −1, −2, −3) to distinguish organisms recovered from polymicrobial cultures.
5. Replicate Identifier [ ]
    - For every isolate submitted the laboratory must prepare **two duplicate vials**, labelled:
        - **Replicate A**
        - **Replicate B**

**Examples:**

- Core isolate:
    - AUJHH-00027 | 14/02/2026 | SAU | 26-00845-1 | A
    - AUJHH-00027 | 14/02/2026 | SAU | 26-00845-1 | B
- Polymicrobial sample (two organisms):
    - AUJHH-00027 | 03/05/2026 | ECO | 26-11234-1 | A
    - AUJHH-00027 | 03/05/2026 | ECO | 26-11234-1 | B
    - AUJHH-00027 | 03/05/2026 | SAU | 26-11234-2 | A
    - AUJHH-00027 | 03/05/2026 | SAU | 26-11234-2 | B

Where: ECO = local organism code for E. coli and SAU= S. aureus and A/B = duplicate vials.

## 9. SUPPLEMENTARY ISOLATE REPOSITORY

Some laboratories routinely freeze down PJI isolates and / or have the capacity to store more than a single isolate. In this case repeat or multiple isolates can be submitted to the ROADMAP isolate repository.

These will be classed either as a "repeat isolates" (isolates of the same species collected ≥ 30 days after the index isolate), or as **multiple isolates** (of different organisms) from polymicrobial PJI or if fungi.

Collection of repeat isolates may be used for accurate differentiation between relapse and reinfection[3,4] as well as to investigate causes of treatment failure[5]. **Storage of isolates for the 'Supplementary Repository' (see Table 1) is optional.** As in the core repository, selection and storage of supplementary isolates, should be directed by the site PI using the guiding framework presented in **Section 8.3**.

Please note that the ROADMAP trial has the capacity to reimburse laboratories for storage costs of **only one (1) isolate (in duplicate) per patient episode** (covered by the Regional Repository budget; more

details in **Section 13).** Storage of repeat or multiple isolates is at the responsibility of the **regional coordinating centre and / or site** and will not be reimbursed by the central trial budget.

**Table 1:** Isolate Storage

| **Core Isolate Repository** | **Supplementary Isolate Repository**<br>*Participation is optional although all laboratories are encouraged to participate if feasible.* |
|---|---|
| One (1) representative bacterial isolate per patient episode | One (1) representative isolate of <u>all</u> identified species and antibiotic susceptibility profiles (deemed clinically relevant). This includes fungi.<br><br>Subsequent or repeat isolates (≥ 30 days) |

## *9.1 Labelling Requirements for the Supplementary isolates*

To ensure consistent traceability across sites — particularly where:

- participants have polymicrobial infections,
- laboratories provide several isolates per sample, and
- repeat isolates are received >30 days later,

each isolate must be labelled individually and provided in duplicate vials (A and B).

Each vial must include the same five components as indicated in **Section 8.7.1**.

ROADMAP ID | COLLECTION DATE | ORGANISM CODE | LAB # | REPLICATE ID

- Core isolate:
  - AUJHH-00027 | 14/02/2026 | SAU | 26-00845-1 | A
  - AUJHH-00027 | 14/02/2026 | SAU | 26-00845-1 | B
- Repeat isolate (>30 days later):
  - AUJHH-00027 | 12/06/2026 | SAU | 26-14567-1 | A
  - AUJHH-00027 | 12/06/2026 | SAU | 26-14567-1 | B

Where: ECO = local organism code for E. coli and SAU= S. aureus and A/B = duplicate vials.

## *9.2 Labelling of Supplementary Isolates (Variant Identifier)*

In some cases, more than one isolate of the same organism may be recovered from a single specimen (e.g. isolates with differing antibiograms or other phenotypic characteristics). To ensure unambiguous identification and traceability, each such isolate must be assigned a **Variant Identifier**.

The Variant Identifier [ ] is a sequential suffix applied **only when multiple isolates of the same species are derived from the same specimen** and should be recorded as **V1, V2, V3**, etc.

The Variant Identifier must:

- Be included on the isolate vial label
- Be recorded in the specimen transport log and relevant database fields
- Be used consistently across all documentation relating to that isolate

The Variant Identifier does not imply genetic relatedness or resistance mechanism and is used solely for differentiation and tracking purposes.

**Example:**

ROADMAP ID | COLLECTION DATE | ORGANISM CODE | LAB # | REPLICATE ID | VARIANT IDENTIFIER

- AUJHH-00027 | 14/02/2026 | SAU | 26-00845-1 | A | V1
- AUJHH-00027 | 14/02/2026 | SAU | 26-00845-1 | B | V1
- AUJHH-00027 | 14/02/2026 | SAU | 26-00845-1 | A | V2
- AUJHH-00027 | 14/02/2026 | SAU | 26-00845-1 | B | V2

### *9.*3 Submission Guidance:

- Supplementary isolates are optional, and should be prioritised based on:
  - clinical relevance,
  - PI discretion,
  - laboratory capacity.
- Guidance for Laboratories Handling Polymicrobial or Longitudinal Samples
  - Some laboratories assign sub-labels such as -1, -2, -3 for organisms grown from a single specimen; all such organisms may be submitted if they are clinically relevant.
    - **Example:** A single surgical specimen with polymicrobial growth (E. coli and S. aureus) → Two organisms x 2 vials each = four vials to submit.
  - If a participant has a follow-up procedure: Repeat isolates collected >30 days after the initial PJI episode should be sent (again, in duplicate).

## 10. TRANSFER AND RECALL SCHEDULE

At **6 – to - 12-month** intervals, the regional management group and regional trial steering committee will facilitate the transfer of all stored isolates (including the duplicate) in batches to the **Regional Repository site**. The **regional coordinating centre will be responsible for arranging and funding all transport**

**costs** associated with these regional transfers. At **2 – 4-year** intervals, **a complete set of isolates (excluding the duplicate) and their associated metadata** will be recalled from each Regional Repository to the **Global isolate Repository** at HMRI (Australia), under the direction the of ROADMAP Repository Coordinator. All **transport costs for global recalls will be covered centrally by the ROADMAP trial**.

As part of each recall (regional and global), the Repository Coordinator will provide each Pathology laboratory/Regional Repository with a prefilled Specimen Transport Log (see **Section 17, Appendix 17.2**). This form must be completed by:

- adding the senders contact details, including local laboratory accession number,
- documenting the date of collection for each isolate,
- logging the participant study ID number for each specimen and
- confirming (by a tick) all ROADMAP PJI isolates have been included in the shipment.

The designated site delegate will work closely with the local laboratory to ensure all PJI isolates are accounted for and prepared for transport. Once completed, an electronic copy of the specimen transport log must be emailed to both the Regional and Global Repository Coordinator (isolate_repository@roadmaptrial.com) and the Central ROADMAP TMG (tmg@roadmaptrial.com.au). In addition, a signed hard copy must accompany the shipment.

Shipments will be coordinated by the appropriate ROADMAP Repository Coordinator and dispatched to either the Regional Repository or the Global Coordinating Centre at the Hunter Medical Research Institute (HMRI) in Australia. All shipments will be sent using an approved pre-paid courier service. Isolates and accompanying documentation will flow from trial sites to their designated Regional Repositories, and subsequently to the Global Repository at HMRI (see **Figure 1**).

### *10.1 Vial Duplication and Distribution Requirements*

- **Australia:** For every isolate submitted—whether part of the core repository or the supplementary repository—two duplicate vials (Replicates A and B) must be prepared and provided.
- **International Sites:**
  Replicate A should be shipped to the Global Repository.
  Replicate B should be stored locally at the Regional Repository.
- **Exception:** If a Regional Repository does not have adequate long-term storage capacity, both Replicate A and Replicate B may be sent directly to the Global Repository.

# 11. PREPARATION FOR ISOLATE TRANSPORT

## *11.1 General Principles*

All isolate shipments must be **transported on dry ice** to ensure they remain frozen throughout transit. The quantity of dry ice included should be sufficient to maintain the required storage temperature (−80°C) for the expected duration of transport, with allowance for possible courier delays. As a general guide, approximately **5 - 10 kg (10 - 20 lbs) of dry ice is recommended per 24 hours of transit**, with adjustments made based on the quality of insulation used.

All shipments must comply with International Air Transport Association (IATA) packing instruction 650 for Biological Substances, Category B (UN3373), and IATA packing instruction 954 for dry ice (UN1845). Compliance with these instructions requires the use of the triple-packaging system and adherence to all labelling and documentation requirements (See **Figure 4**). The outer package must clearly display both UN3373 and UN1845 hazard labels, as well as the net weight of dry ice contained within (See **Figure 4**).

Personnel preparing shipments must be **appropriately trained** in these packaging and documentation procedures, including the **safe handling of dry ice**. For sites shipping isolates from **outside Australia**, an **export permit** may also be required. A detailed breakdown of packing materials and labelling requirements is provided in **Section 11.3.1**.

Sites and Regional Repository are asked to avoid sending shipments that may arrive over a weekend, as trial staff availability is limited during this time, increasing the risk of delays, inappropriate storage, and / or compromise of sample integrity.

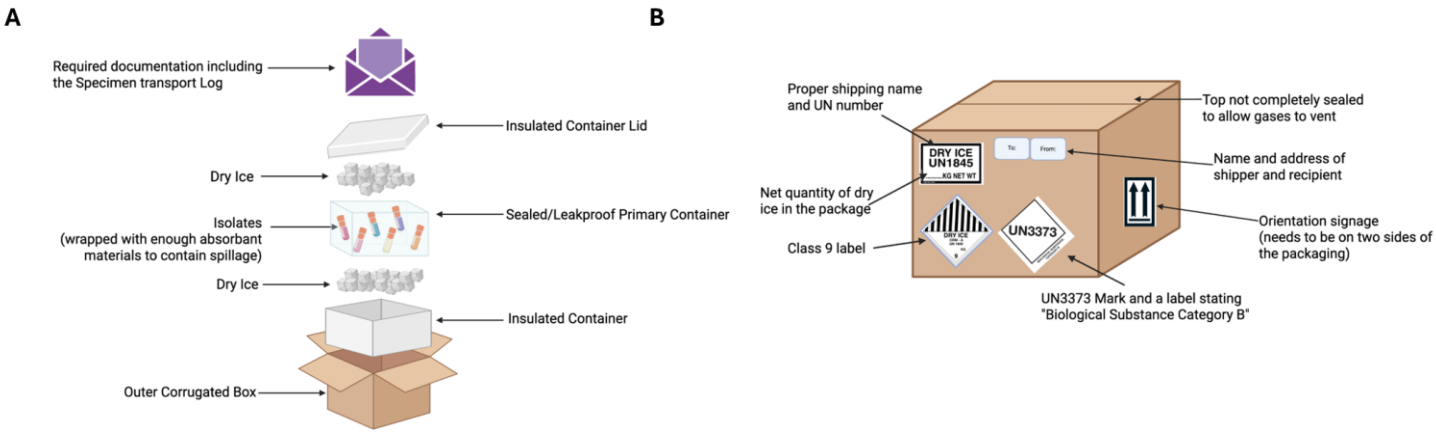


**Figure 4: Packaging and labelling of isolates shipped on dry ice. (A)** shows the internal packaging configuration: isolates are sealed in a primary container, surrounded by dry ice, and placed in an insulated container, which is then enclosed in an outer corrugated box. **(B)** Illustrates the required outer box labelling, including proper shipping name and United Nations (UN) number, net quantity of dry ice, Class 9 hazard label, shipper / recipient details, ventilation allowance, and orientation markings.

## *11.2 Labelling and Shipping Address*

The isolates need to be labelled for transport with the as indicated in **Section 8.7.1**.

**Figure 5** provides the designated Global Repository courier shipment address, which **must be used exactly as listed to align with the import permit held at HMRI**. A completed copy of the Specimen Transport Log must be placed with the bacterial isolates for transport, and an electronic copy of the log should be emailed in advance to the Regional and Global Repository Coordinators, together with notification of the pending shipment.

For any queries or assistance regarding the shipment process, please contact the Global Repository Coordinator (isolate_repository@roadmaptrial.com) and/or the ROADMAP Trial Management Team (tmg@roadmaptrial.com.au).

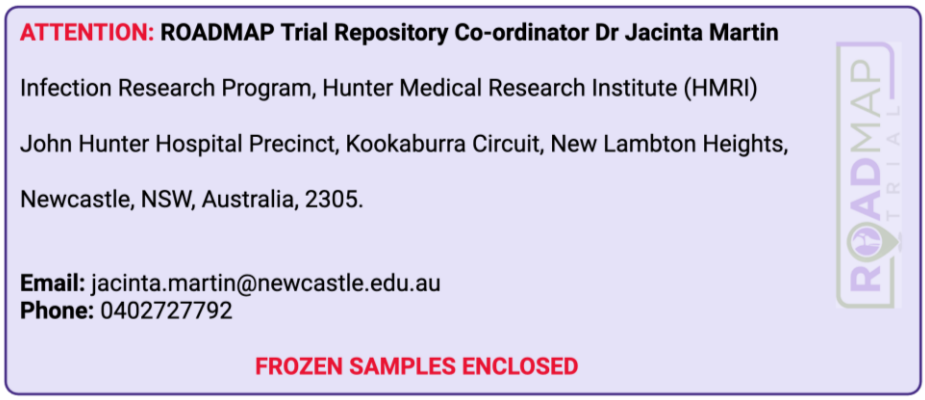


**Figure 5 Courier Shipment Address.**

## *11.3 International Air Transport Association (IATA) Packing Requirements*

To comply with IATA packing instruction 650 for Biological Substances, Category B (UN3373) and IATA packing instruction 954 for dry ice (UN1845) each shipment box should be constructed from sturdy fibreboard (corrugated cardboard) to withstand handling during transport. Inside, a non-airtight insulated liner, such as polystyrene foam, should be used to maintain cold temperatures. Isolates should be placed in a sealed primary container and arranged securely within the insulated cavity so they cannot shift or break during transit. This primary container must also be surrounded by enough absorbent material to contain spillage. Dry ice should then be added around or above and below the isolates, ensuring adequate space is left for the sublimated gas to vent safely (**Figure 4A**). The outer box must be closed firmly but not completely sealed, to allow pressure / sublimated gasses to vent. All seams should be reinforced with strong packing tape to keep the box intact during transport, and the outer packaging must be clearly labelled in accordance with dry ice and biological substance transport regulations (See **Figure 4B**; see breakdown provided in **Section 11.3.1**).

### 11.3.1 Quick guide of IATA Packing Requirements

A quick guide of the IATA compliant packing requirements is detailed below:

**1. Triple Packaging System**

- **Primary receptacle**
  - Leakproof container(s) containing the specimen (e.g., tubes, vials).
  - Must be securely sealed and appropriately labelled.
  - Each primary container must be wrapped with enough absorbent material to absorb the entire contents in case of leakage.
- **Secondary packaging**
  - A leakproof, durable container (e.g., sealed plastic canister or zip-lock pouch).
  - Multiple primary receptacles may be placed in one secondary package if individually wrapped to prevent contact.
- **Outer packaging**
  - A strong, rigid box (minimum dimension of 100 mm × 100 mm).
  - Must protect the secondary container(s) and withstand typical transport stresses.

**2. Markings and Labelling**

- **UN3373 mark:** A diamond-shaped mark with "UN3373" printed inside.
- **Proper shipping name:** "Biological Substance, Category B."
- **Shipper and consignee details**: Name, address, and contact information of both.
- **Orientation arrows:** If liquids are included, arrows must be placed on two opposite sides of the package.
- **For dry ice:** Class 9 hazard label (black and white stripes, diamond-shaped) and "UN1845" must be clearly marked on the package.
  - Net weight of dry ice in kilograms must be indicated (e.g., "Dry Ice, UN1845, 5 kg").

**3. Quantity Limits**

- **For air/ground transport:**
  - Each primary receptacle ≤ 1 L.
  - Total per outer package ≤ 4 L (for liquids).
  - For solids, the total per outer package ≤ 4 kg.
- **For dry ice:**
  - No absolute maximum per package, but there are airline/operator-specific limits often apply.

**4. Dry Ice**

- Dry ice is regulated separately as UN1845, Class 9.

- Dry ice must not be sealed in airtight containers, because it sublimates into carbon dioxide ($CO_2$) gas and could rupture the package.
- Place dry ice outside the secondary container (never in direct contact with samples) but inside the outer package, with insulation (e.g. polystyrene).
- The outer packaging must allow venting of gas.

**5. Documentation**

- A standard air waybill is required, with:
  - UN3373- Biological Substance, Category B
  - UN1845- Dry Ice and net weight

# 12. RECEIVING AND ACCESSION PROCESS

When a shipment is received at either the Regional or Global Repository, the ROADMAP Repository Coordinator will open the package and cross-check the isolates against the accompanying Specimen Transport Log to ensure all isolates are accounted for and possess accurate labelling and documentation. If any discrepancies are identified, the Repository Coordinator will promptly contact the Regional Repository / site for clarification. Once the shipment has been received and verified, all sites will be notified by email to confirm its arrival. The isolates will then be accessioned into the Regional or Global ROADMAP repository database (RedCap) as appropriate and stored in a -80°C freezer under appropriate long-term conditions until recalled to the Global Repository or required for future analyses.

- **Note on Sample Handling:** Upon receipt by the Global ROADMAP Repository, isolates will not be sub-cultured, nor will species identity or viability be confirmed. Specimens will only be catalogued and frozen for long-term preservation. These processes — including sub-culturing, viability assessment, and species confirmation — will need to be undertaken as part of future sub-studies involving the use of these isolates.

## *12.1 Accessioning workflow*

The following information contributes part of a broader receiving and accession workflow at HMRI, where pre-arrival preparation, shipment tracking, and allocation of trained staff help ensure shipments are processed efficiently and in line with repository requirements.

### 12.1.1 Pre-Arrival Preparation

- **Notification:** The Repository Coordinator should receive advance notice (with electronic transport log attached).

- **Shipment tracking:** Use courier tracking to anticipate arrival and ensure staff availability (avoid weekend deliveries)
- **Designated receiving staff:** Ensure trained staff are scheduled to handle arrival, unpacking, and accession.

### 12.1.2 Initial Receiving

- **Inspection of package:** Check outer packaging for damage, correct UN3373/UN1845 labelling, and dry ice weight labelling.
- Record courier details (arrival time, condition).
- **Temperature check:** Confirm sufficient dry ice remains (or note if dry ice is depleted).
- **Documentation check:** Ensure a hard copy of the Specimen Transport Log is included

### 12.1.3 Cross-Checking

- Match shipment contents against the Specimen Transport Log (see **Section 17, Appendix 17.2**)
- **Discrepancies:** Recorded immediately and notify sites / Regional Repository coordinating teams

### 12.1.4 Accessioning in REDCap

- Assign a central repository accession number (unique ROADMAP ID).
- Enter isolate details into the ROADMAP Repository Database:
    - Participant Study Number
    - Country of Origin (of sample)
    - Site code (e.g. AUJHH)
    - Laboratory Isolate/Accession Number
    - Specimen Source/Location (e.g. intraoperative tissue, blood cultures or intraoperative sterile swabs)
    - Collection date (DD/MM/YYYY)
    - Isolate number (if applicable)
    - Organism Code
    - Form of specimen (e.g. frozen media, swabs etc)
    - Duplicates received (yes/no)
    - Date package sent (DD/MM/YYYY)
    - Date package received (DD/MM/YYYY)
    - Condition on arrival (adequate, compromised, lost dry ice, etc.)
    - Storage location (freezer, shelf, rack, box, inner position (e.g. A1, A2))
- Link scanned copy of the Specimen Transport Log to the electronic record.

### 12.1.5 Storage

- Transfer isolates into long-term storage (−80 °C freezer).

- Place isolates in a labelled storage box / rack with position recorded in the RedCap database for future retrieval.

### 12.1.6 Notification Back to Site

- Send confirmation email to the site / regional repository coordination team and TMG:
    - Shipment received
    - All isolates logged and accessioned (or list discrepancies / issues)

### 12.1.7 Quality Assurance

- Regular audits of database entries vs. physical inventory.
- Annual reconciliation to confirm no missing or mislabelled samples.
- Flag isolates with issues (e.g., thawed, broken vial or incomplete documentation).

## 13. RENUMERATION

Each participating ROADMAP region will host a Regional isolate Repository and will therefore be responsible for the local storage of PJI isolates collected from enrolled platform participants within their geographical region. The regional management group, together with the regional trial steering committee, will coordinate the activities of the Regional Repository and will retain ownership of it. This includes **responsibility for organising and funding the collection and shipment of isolates from participating site laboratories to the Regional Repository at intervals of approximately every 6 – 12 months.**

**Australia:** ROADMAP can reimburse **Australian sites** for the storage of **one (1) isolate in duplicate per participant episode**. Storage of any additional isolates (repeat or multiple) is at **the discretion and cost of the site**, not the central ROADMAP budget.

**International Sites (New Zealand):** Isolate costs are covered in the site participant payment reimbursement (i.e. it is all inclusive payment).

**International Sites (other):** International regional repositories will manage and reimburse storage for **one (1) isolate in duplicate per participant episode** according to local agreements. The ROADMAP central team will cover **international shipping costs only,** not storage for core or supplementary isolates.

## 14. RIFAMPCIN TESTING AND RESISTANCE

***Note:*** Clinical laboratories are not expected to test for rifampicin susceptibility if it is not part of their routine workflow for the organism(s) identified. This section has been included to inform central retrospective lab testing.

We recommend using the European Committee on Antimicrobial Susceptibility Testing (EUCAST)[6] interpretative criteria for *Staphylococcus* spp, *Streptococcus* spp and *Corynebacterium* spp. No interpretative criteria are available for *Enterococcus* spp and *Cutibacterium* spp (see **Table 2** below).

**Table 2:** European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints for rifampicin resistance in Gram positive microorganisms (Version 14.0, valid 2024-01-01). N/A refers to no available. * These breakpoints can be applied to viridans streptococci. However, EUCAST suggests reporting as "devoid of rifampicin resistance mechanisms but not as susceptible to rifampicin".

| **Microorganism** | **Minimum Inhibitory Concentration (MIC) (mg/L)** | **Zone diameter (mm)** | **Disk content (ug)** |
|---|---|---|---|
| *Staphylococcus aureus* | ≤ 0.06 | ≥ 26 | 5 |
| *Coagulase-negative staphylococci* | ≤ 0.06 | ≥ 30 | 5 |
| *Streptococcus group A, B, C, G** | ≤ 0.25 | ≥ 21 | 5 |
| *Streptococcus pneumoniae* | ≤ 0.125 | ≥ 22 | 5 |
| *Enterococcus* | N/A | N/A | N/A |
| *Cutibacterium* | N/A | N/A | N/A |

# 15. FUTURE USE OF REPOSITORY / ISOLATES AND DATA SHARING PRINCIPLES

## *15.1 Ownership*

Each region or site retains ownership of its bacterial isolates. If isolates are shared with other parties such as for sub-studies, a Material Transfer Agreement (MTA) may be required and should outline the rights and responsibilities of the sending and receiving institutions. In general, the sending parties will be informed of any assays, sub-studies, or analyses proposed on ROADMAP isolates, and they will be appropriately recognised in any resulting publications. Isolates should only be shared with third parties with the prior knowledge and consent of both the sending and receiving parties. In addition, any such **studies will need to be endorsed by the ROADMAP MWG and approved by the GTSC** and may, in some instances, require additional consent (e.g. if clinical samples instead of bacterial isolates are collected). Requests to use isolates will be communicated to the relevant regional management team(s). Each region retains the right to decline the use of its isolates for specific purposes.

## *15.2 Scope of Work with the PJI Isolates*

Collected repository isolates will be used for ROADMAP quality control purposes and will be used to inform / facilitate future microbiology sub-studies. Such investigations may include, but are not limited to, bacterial genomics for differentiation between re-infections and relapses as done for the ARREST trial[4]

and central rifampicin susceptibility testing (see **Section 14** for more details). Microbiology sub-studies may also include investigations of correlations between bacterial phenotype (e.g. small colony variants) and genotype with clinical outcomes, testing novel antibiotics or pathophysiology models *in-vitro* and trial quality control testing.

The types of assays that may be performed are indicated below. The assays listed below may be performed at multiple laboratories; however, efforts should be made to harmonise methods to enable inter-laboratory comparisons. In addition, laboratories should ensure that resources are used efficiently to avoid unnecessary duplication and to maximise output.

- Standard antimicrobial susceptibility testing
- Whole genome sequencing
- Other sub-study works that are approved of by the GTSC and MWG

**Note:** This list is a guide and is not meant to be exhaustive.

## *15.3 Application*

The collection of PJI isolates linked to clinical trial data is a valuable resource. We hope that these resources may be accessed by the ROADMAP and broader PJI research community to address clinically and biologically significant questions.

Researchers / clinicians wishing to engage with the repository or apply for access to stored samples must submit a formal written request (an expression of interest or EOI) outlining the intended use and scientific rationale, and ethical approvals using the appended EOI Form (see **Section 17, Appendix 17.2**). All applications will be reviewed by the ROADMAP GTSC, and ROADMAP MWG and Regional Steering Committees in accordance with repository governance procedures to ensure responsible and equitable use of samples.

For further information and submission of your application, please email the ROADMAP Trial Management Team on tmg@roadmaptrial.com with the following subject line **"REPOSITORY SAMPLE ACCESS REQUEST".** Requests for repository samples will typically be reviewed within two (2) weeks of submission, with a decision regarding approval normally made within eight (8) weeks. This timeline allows for consideration by the GTSC and accommodates any required rounds of review. Applicants will be notified of the outcome promptly thereafter.

Further information can be found on the sub-study operating procedure document. Some key definitions are provided below for convenience.

### 15.3.1 Sub-Study Categories

Proposed studies will be categorised as:

- **New Domain:** Uses existing ROADMAP core protocol, statistical appendix, statistical model, infrastructure, and database. Will require an ethics and consent amendment, a domain specific appendix, and additional funding. *Full sub-study review process will need to be completed (See sub-study SOP).*
- **Integrated Clinical Trials:** Includes randomisation within the ROADMAP platform but only applies to a defined subset of ROADMAP participants. Will require addition to ethics, consent and domain specific appendices, and likely additional funding. May have own database and statistical model/analysis plan. *Full sub-study review process will need to be completed (See sub-study SOP).*
- **Prospective Sub-Studies:** May include additional collection of samples or clinical data. Will require sub-study protocols, ethics, and consent for additional study procedures (which may be broadly covered by approval for biobanking of samples), and likely additional funding (which may be provided in-kind by sub-study investigators). *Full sub-study review process will need to be completed (See sub-study SOP).*
- **Pre-Planned Analyses:** Makes use of samples (bacterial isolates) or data that are already being collected. Will require a brief protocol but may or may not require additional ethics or consent, and does not require any change to the CRFs, database or ROADMAP protocol documents. Additional funding may be required, which may be provided in-kind by sub-study investigators. *This type of proposal requires sign-off by the GTSC (GTSC) but may not need to go through the full sub-study review process.*
- **Use of ROADMAP data for harmonised external studies:** An existing study with its own existing protocol and ethics approval (or waiver), and its own data collection/dataset. Requires an extract from the ROADMAP dataset of selected datapoints to integrate with their existing study. *This type of proposal requires sign-off by the GTSC but may not need to go through the full sub-study review process and may have different authorship implications to the other categories.*

### 15.3.2 Sub-Study Review Criteria

Sub-studies will be judged on the following criteria for integration into the ROADMAP Trial. If a sub-study does not fulfil the criteria below, the proposal may be recommended for co-enrolment, data sharing, or meta-analysis.

- Trial Integrity
- Harmony with ROADMAP Protocol
- Adoption of the Pragmatic Trial Approach
- Feasibility
- Global Application
- Funding
- Sample Collection, Analysis & Shipping

The GGTSC will review all sub-study proposals to determine if the sub-study is suitable for integration into the ROADMAP Trial. The sub-studies will be judged on the criteria above, as well as overall scientific merit.

Sub-studies will be judged in 2 stages:

1. Expression of Interest – 1-5 pages (**Section 17, Appendix 17.2**)
2. Full Protocol (see Sub-study appendix for more information)

At each of these stages, the GGTSC will be provided with the opportunity to raise questions and comments for the sub-study lead(s) to respond to, prior to proceeding to the next stage. All comments/questions will be returned to the sub-study lead(s) following the GTSC review.

The EOI (**Section 17, Appendix 17.2**) is the first stage of the review process for all sub studies. This simple, 1–5-page outline will be assessed for scientific merit (including significance and impact, and quality of methods) and feasibility (including available support, sample size, data collection methods, and funding). The EOI will be presented by a GTSC representative at the next suitable GTSC meeting, and any comments or questions will be returned to the sub-study lead(s).

At the end of the GTSC Meeting, the committee will provide a consensus vote for:

- The sub-study to proceed to full protocol
- The sub-study lead(s) to revise the EOI and/or provide a response to queries raised
- The sub-study does not proceed further at this time

### *15.4 Data Sharing*

In general, we foster a culture of open sharing of data and results in the ROADMAP community. Data generated from ROADMAP related isolates should be made available to regional sponsors (the original custodians of the isolates) on reasonable request. This includes data relating to raw and curated whole genome sequence reads.

ROADMAP collaborators may propose sub-studies that make use of data generated from ROADMAP involved laboratories (i.e., not restricted to their own region, site or laboratory). These sub-studies will be assessed and prioritised by the GTSC as per guidance for ROADMAP sub-studies. Those who have generated the data should be willing to share their data on reasonable request and with assurance of involvement in outputs and publications arising thereafter.

See the following documents for more information about data access and sharing for ROADMAP sub-studies:

- Data Sharing Policy
- Authorship and Publication Policy
- Data Management Plan

## 16. REFERENCES


1. McNally M, Sousa R, Wouthuyzen-Bakker M, et al. The EBJIS definition of periprosthetic joint infection. Bone Joint J 2021;103-b(1):18-25. (In eng). DOI: 10.1302/0301-620x.103b1.Bjj-2020-1381.R1.
2. Atkins BL, Athanasou N, Deeks JJ, et al. Prospective evaluation of criteria for microbiological diagnosis of prosthetic-joint infection at revision arthroplasty. The OSIRIS Collaborative Study Group. J Clin Microbiol 1998;36(10):2932-9. (In eng). DOI: 10.1128/jcm.36.10.2932-2939.1998.
3. Pham TT, Lazarevic V, Gaia N, et al. Second Periprosthetic Joint Infection Caused by Streptococcus dysgalactiae: How Genomic Sequencing Can Help Defining the Best Therapeutic Strategy. Front Med (Lausanne) 2020;7:53. (In eng). DOI: 10.3389/fmed.2020.00053.
4. Thwaites GE, Scarborough M, Szubert A, et al. Adjunctive rifampicin for Staphylococcus aureus bacteraemia (ARREST): a multicentre, randomised, double-blind, placebo-controlled trial. The Lancet 2018;391(10121):668-678. DOI: 10.1016/S0140-6736(17)32456-X.
5. Giulieri SG, Leroi M, Daniel D, et al. A multi-hospital, clinician-initiated bacterial genomics programme to investigate treatment failure in severe Staphylococcus aureus infections. Nature Communications 2025;16(1):4869. DOI: 10.1038/s41467-025-60045-4.
6. Testing ECoAS. Clinical breakpoints and dosing of antibiotics. EUCAST. (https://www.eucast.org/clinical_breakpoints).

# 17. APPENDIX

## *17.1 Organism List (STANDARD 3-LETTER ORGANISM CODES)*

Organism codes reflect standard NSW Health / Australian hospital microbiology conventions commonly used at John Hunter Hospital. Sites may use local laboratory codes where these differ; a copy of the local organism code list must be provided to the Biobank Coordinator for reference.

### *Gram-Positive Bacteria*

| **Organism** | **3-Letter Code** |
|---|---|
| Staphylococcus aureus | SAU |
| Coagulase-negative Staphylococcus spp. | CNS |
| Staphylococcus epidermidis | SEP |
| Staphylococcus lugdunensis | SLU |
| Enterococcus faecalis | EFA |
| Enterococcus faecium | EFM |
| Streptococcus pyogenes (Group A) | GAS |
| Streptococcus agalactiae (Group B) | GBS |
| Streptococcus pneumoniae | SPN |
| Viridans group streptococci | VGS |
| Cutibacterium acnes | CAC |
| Listeria monocytogenes | LMO |

### *Gram-Negative Bacteria (Enterobacterales)*

| **Organism** | **3-Letter Code** |
|---|---|
| Escherichia coli | ECO |
| Klebsiella pneumoniae | KPN |
| Klebsiella oxytoca | KOX |
| Enterobacter cloacae complex | ECL |
| Serratia marcescens | SMA |
| Proteus mirabilis | PMI |
| Proteus vulgaris | PVU |
| Morganella morganii | MMO |

| | |
|---|---|
| Citrobacter freundii | CFR |
| Citrobacter koseri | CKO |

### *Non-Fermenting Gram-Negative Bacteria*

| **Organism** | **3-Letter Code** |
|---|---|
| Pseudomonas aeruginosa | PAE |
| Acinetobacter baumannii | ABA |
| Stenotrophomonas maltophilia | SMA |
| Burkholderia cepacia complex | BCC |
| Achromobacter spp. | ACH |

### *Anaerobes*

| **Organism** | **3-Letter Code** |
|---|---|
| Bacteroides fragilis | BFR |
| Clostridioides difficile | CDI |
| Clostridium perfringens | CPR |
| Anaerobic Gram-positive cocci | AGC |
| Anaerobic Gram-negative rods | AGR |

### *Fungi / Yeasts*

| **Organism** | **3-Letter Code** |
|---|---|
| Candida albicans | CAL |
| Candida glabrata | CGL |
| Candida parapsilosis | CPA |
| Candida tropicalis | CTR |
| Candida krusei | CKR |
| Candida spp. (unspecified) | CAN |
| Aspergillus spp. | ASP |

### *Other / Common Groupings*

| **Organism** | **3-Letter Code** |
|---|---|

| | |
|---|---|
| Mixed anaerobes | MIX |
| Gram-positive cocci (unspecified) | GPC |
| Gram-negative rods (unspecified) | GNR |
| No growth | NOG |

### *17.1 Specimen Transport Log*

**Site Code:** _____
**Regional Repository/ Country:** _____
**Prepared by:** _____
**Date:** ____ / ____ / _____

---

***Specimen Details (Multiple Specimens per Shipment)*** **-Use one row per specimen. Add rows as required.**

| Participant Study Number | Collection Date (DD/MM/YYYY) | Organism Code | Specimen Source / Location | Isolate No. | Lab Accession No. | Replicate No. | Packed |
|---|---|---|---|---|---|---|---|
| AUXXX-00027 | 08/04/1992 | ECO | Synovial Fluid | 1 | 123456789 | 1 | ✓ |
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| Participant Study Number | Collection Date (DD/MM/YYYY) | Organism Code | Specimen Source / Location | Isolate No. | Lab Accession No. | Replicate No. | Packed |
|---|---|---|---|---|---|---|---|
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*Transport Details (Per Shipment)*

| Field | Entry |
|---|---|
| **Date Package Sent (DD/MM/YYYY)** | |
| **Courier / Transport Method** | |
| **Tracking Number (if applicable)** | |

**Packed and Verified By:** _____

**Signature:** _____

**Date:** ____ / ____ / _____

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### *Storage Details (on Receipt)* Internal Use Only

| Field | Entry |
|---|---|
| **Date Package Received (DD/MM/YYYY)** | |
| **Storage Facility** | |
| **Freezer / Shelf / Rack** | |
| **Box Number** | |
| **Inner Position (e.g. A1, A2)** | |
| **Date Placed into Storage (DD/MM/YYYY)** | |
| **Stored By (Initials)** | |
| **Condition on Arrival (e.g. adequate, compromised, lost dry ice)** | |

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### *Documentation and Records*

☐ Scanned copy of this Specimen Transport Log uploaded

☐ Signed hard copy to be included with samples

### *Notes / Comments*

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**Checked and Verified By:** _____

**Signature:** _____

**Date:** ____ / ____ / _____

## *17.2 Isolate Application Form/ Sub-study Expression of interest (EOI)*

**ROADMAP Sub-study Expression of Interest (EOI)**

An Expression of Interest (EOI) is the first stage of the review process for all sub studies (including use of the isolate repository). The sub-study lead(s) will be required to complete the sub-study EOI template and return the completed form to the ROADMAP Management Team (TMG@roadmaptrial.com).

The EOI will be presented by a GTSC representative at the next suitable GTSC meeting, and any comments or questions will be returned to the sub-study lead(s).

| 1. **SUBSTUDY INVESTIGATOR DETAILS** | |
|---|---|
| Name of Project Leader *(lead person)* | |
| Email Address | |
| Institution & Region | |
| Other Sub-Study Collaborators | |
| Do you or any sub-study collaborators have any conflicts of interest to declare? | Yes No |
| **If yes,** please state details of the perceived conflict(s) | |
| Does the planned sub-study involve students? | Yes No |

| 2. **SUBSTUDY PROJECT DETAILS** | |
|---|---|
| Title of project: | |
| Proposed Short Title of Project *(if available)*: | |
| Proposed Sub-Study Category<br>*(See Substudy SOP for further detail)* | New domain<br>Integrated Clinical Trial<br>Prospective Sub-Study<br>Pre-Planned Analysis |

| | |
|---|---|
| | Use of ROADMAP Data for Harmonised External Study |
| Proposed Regions | Any regions interested in participating<br><br>Specific Regions *(please specify):* |
| ROADMAP Enrolment | Platform Patients Only<br><br>Registry Patients Only<br><br>Both Platform AND Registry Patients<br><br>Divert Patients from ROADMAP |
| ROADMAP Participants (select all that apply) | Adults<br><br>other specific sub-study participant<br><br>please specify _____ |
| Timeline for the project:<br><br>*(Include recruiting start and data collection finish dates)* | |

| 3. **SUB-STUDY PROPOSAL OUTLINE (no more than 700 words)** |
|---|
| Use the following headings in the order below:<br><br>1. **Background and Rationale**<br>2. **Hypothesis/research questions**<br>3. **Methods**<br>4. **Project Endpoints/Outcomes**<br>5. **What data points and/or specimens are required or brief schedule of events** |

| 4. **SUBSTUDY REVIEW CRITERIA –** ***Please provide a brief justification/response to each of the following points.*** | **GTSC Use Only** |
|---|---|

| | | |
|---|---|---|
| **Trial Integrity**<br>1. Does this sub-study require access to data that could threaten trial integrity?<br>2. Does this sub-study wish to report on trial outcomes prior to the ROADMAP Trial Results paper?<br>3. Does the sub-study wish to report on sub-study outcomes by treatment allocation prior to the ROADMAP Trial Results paper?<br>4. Other potential trial integrity issues? | | |
| **Harmony with ROADMAP Protocol**<br>1. Does the sub-study require a significant amount of data/sample collection outside of standard care or ROADMAP trial protocols?<br>2. Are the sample/data collection harmonised with the ROADMAP core protocol and other ROADMAP sub-studies? | | |
| **Adoption of the Pragmatic Trial Approach**<br>1. Does this sub-study place a significant additional burden on the participant or the site study team?<br>2. Does the proposal include only the necessary data collection to answer the research question? | | |
| **Feasibility**<br>1. Is the sample size reasonable and achievable?<br>2. Will the research question be able to be answered recruiting only ROADMAP Trial participants? | | |
| **Global Application**<br>1. Has the investigator considered the ability for the sub-study to be conducted in other regions/countries? | | |

| | | |
|---|---|---|
| 2. Is the protocol applicable to other countries/regions? | | |
| **Funding**<br>1. Has funding for this sub study been considered or obtained?<br>2. Are there any grants the investigator is intending to apply for to cover the costs of the sub-study?<br>3. Has a proposal been put forward to the central team for covering some or all of the costs? | | |
| **Sample Collection, Analysis & Shipping**<br>1. Will samples be collected for this sub study and shipped to an external location?<br>2. Has the shipping and storage of theses samples been considered?<br>3. Has a plan for the analysis been generated? | | |

| 5. **GTSC REVIEW (GTSC USE ONLY)** | |
|---|---|
| Date of GTSC Review: | |
| GTSC Decision: | Proceed to Detailed Proposal<br>Proceed to Full Protocol<br>Revise EOI & Respond to GTSC Comments *(Section 6 below)*<br>Not proceed further at this time |
| GTSC Queries for Response *(if applicable):* | |

| 6. **RESPONSE TO QUERIES *(please complete this section upon resubmission of your EOI if required)*** | |
|---|---|
| Query 1: | Query 1 Response: |
| Query 2 (repeat as required): | Query 2 Response (repeat as required): |