Australian Government
Department of Health, Disability and Ageing
Office of the Gene Technology Regulator

November 2025

Risk Assessment and Risk Management Plan (Consultation version) for

DIR-221

Clinical trial of a genetically modified *Escherichia coli* for the treatment of ulcerative colitis

Applicant: Melius MicroBiomics Pty Ltd

This RARMP is open for consultation until 14 January 2026

Written comments on the risks to human health and safety and the environment posed by this proposed clinical trial of the GM *E. coli* treatment are invited. You may make your submission

Via the consultation hub: https://consultations.health.gov.au/ogtr/dir-221-consultation

via mail to: The Office of the Gene Technology Regulator,
MDP 54 GPO Box 9848, Canberra ACT 2601

or via email to: ogtr@health.gov.au.

Please note that issues regarding patient safety and the quality of the therapeutic **do not** fall within the scope of these evaluations as they are the responsibilities of other agencies and authorities.

Summary of the Risk Assessment and Risk Management Plan

(Consultation Version) for

Licence Application DIR-221

Introduction

The Gene Technology Regulator (the Regulator) has received a licence application to conduct a clinical trial using a genetically modified organism (GMO). It qualifies as a Dealing involving the Intentional Release (DIR) of GMOs into the Australian environment under the *Gene Technology Act 2000*.

The applicant, Melius MicroBiomics Pty Ltd (Melius), proposes to conduct a clinical trial to evaluate the safety and efficacy of a genetically modified (GM) *Escherichia coli* for the treatment of Australian patients with ulcerative colitis. Ulcerative colitis is a type of inflammatory bowel disease (IBD) that causes the formation of sores due to inflammation that affects the lining of the large intestine (colon) and rectum.

Clinical trials in Australia are conducted in accordance with requirements of the *Therapeutic Goods Act 1989*, which is administered by the Therapeutic Goods Administration (TGA). Therefore, in addition to approval by the Regulator, Melius would also require authorisation from TGA before the trial commences. Clinical trials conducted in Australia must also be conducted in accordance with the *National Statement on Ethical Conduct in Human Research* and with the *Guidelines for Good Clinical Practice* of the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use. Melius would also require approval from the Department of Agriculture, Fisheries and Forestry (DAFF) for import of the GMO into Australia.

The Regulator has prepared a Risk Assessment and Risk Management Plan (RARMP) for this application, which concludes that the proposed clinical trial poses negligible risks to human health and safety and the environment. Licence conditions have been drafted for the proposed clinical trial. The Regulator invites submissions on the RARMP, including draft licence conditions, to inform the decision on whether to issue a licence.

The application

Project Title	Clinical trial with genetically modified *Escherichia Coli* for the treatment of ulcerative colitis.
Parent organism	*Escherichia coli* (Nissle strain).

Genetic modifications	*E. coli* has been modified by the:
	• Insertion of 2 copies of the tetrathionate reductase (*ttr*) operon from *Salmonella enterica* – survival advantage in inflammatory environment.
	• Deletion of 2 genes[1]
	  ○ Gene A - reduced survivability in the broader environment; and
	  ○ Gene B - potentially reduced ability to colonise the healthy gut compared to wild type (WT)

[1] Confidential Commercial Information: Some details about the modification in GM *E. coli* have been declared as Confidential Commercial Information under section 185 of the Act. This information will be made available to the prescribed experts and agencies that will be consulted on this application. CCI is not available to the public.

Principal purpose	The proposed trial is a Phase 1 study designed to evaluate the safety and efficacy of GM *E. coli*, for the treatment of Australian patients with ulcerative colitis.
Previous clinical trials	None, this is a first in human clinical trial.
Proposed limits and controls	
Proposed duration	5 years
Proposed release size	Up to 36 participants in Australia (including placebo).
Proposed locations	This clinical trial would be conducted within Australia at a hospital or clinical trial sites (medical facilities). The specific clinical trial sites are yet to be identified.
Proposed controls	• The GMO would be administered to trial participants within clinical trial sites. • Staff handling the GMO would be trained and would wear personal protective equipment. • Waste that may contain the GMO would be disposed of via the facility standard practices for disposal of biological waste. • Any unused doses of GMO would be disposed of at the clinical trial site at the end of the trial, in accordance with the *Transport, Storage and Disposal Guidelines*. • Participants would be instructed: ○ on appropriate hygiene practices, including proper hand washing procedures following toilet use. ○ to abstain from unprotected sex and to use a double barrier method. • The GMO would be transported and stored according to *Transport, Storage and Disposal Guidelines*.

Risk assessment

The risk assessment process considers how the genetic modification and proposed activities conducted with the GMO might lead to harm to people or the environment. Risks are characterised in relation to both the seriousness and likelihood of harm, taking into account information in the application (including proposed controls), relevant previous approvals and current scientific/technical knowledge. Both the short- and long-term risks are considered.

Credible pathways to potential harm that were considered include the potential exposure of people and animals to the GMO and the potential for transfer of genetic material to and from the GMO. The potential for the GMO to be released into the environment and its effects was also considered.

The risk assessment concludes that the trial poses negligible risks to human health and safety and to the environment. No specific risk treatment measures are required to manage these negligible risks.

Important factors in reaching the conclusions of the risk assessment included that:

- the parent organism has a long history of safe use as a probiotic,
- the GMO has selective replication in patients with inflammatory bowel disease,

- unintended exposure to the GMO would be minimised by the proposed limits and controls outlined in the draft risk management plan, and
- the likelihood of complementation and recombination of the GMO with other bacteria is unlikely to result in bacteria that is more pathogenic than the parent organism.

Therefore, the Regulator considers that the dealings involved do not pose a significant risk to either people or the environment.

Risk management

The risk management plan describes measures to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan is given effect through licence conditions. Draft licence conditions are detailed in Chapter 4 of the RARMP.

As the level of risk is considered negligible, specific risk treatment is not required. However, since this is a clinical trial, the draft licence includes limits on the number of trial participants and trial duration, with treatment locations limited to hospitals and clinical trial sites, and controls to minimise the potential for the GMO to spread in the environment. Additionally, there are several general conditions relating to ongoing licence holder suitability, auditing and monitoring, and reporting requirements which include an obligation to report any unintended effects.

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Abbreviations

AICIS	Australian Industrial Chemicals Introduction Scheme
APVMA	Australian Pesticides and Veterinary Medicines Authority
CDC	Centers for Disease Control and Prevention
CFU	Colony forming units
CTA	Clinical Trial Approval
CTN	Clinical Trial Notification
DAFF	Department of Agriculture, Fisheries and Forestry
DIR	Dealings Involving Intentional Release
DNA	Deoxyribonucleic acid
EcN	*Escherichia coli* Nissle strain 1917
EU	European Union
FSANZ	Food Standards Australia New Zealand
GTTAC	Gene Technology Technical Advisory Committee
GM	Genetically modified
GMO	Genetically modified organism
HREC	Human Research Ethics Committee
IATA	International Air Transport Association
IBC	Institutional Biosafety Committee
ICH-GCP	International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use – Guidelines for Good Clinical Practice
NATA	National Association of Testing Authorities
NHMRC	National Health and Medical Research Council
NPAAC	National Pathology Accreditation Advisory Council
NSQHS	National Safety and Quality Health Service Standards
OGTR	Office of the Gene Technology Regulator
PPE	Personal Protective Equipment
PCR	Polymerase chain reaction
RAF	Risk Analysis Framework
RARMP	Risk Assessment and Risk Management Plan
SOP	Standard Operating Procedure
the Act	The *Gene Technology Act 2000*
the Regulations	The Gene Technology Regulations 2001
the Regulator	The Gene Technology Regulator
TGA	Therapeutic Goods Administration
TSDs	The Regulator's *Guidelines for Transport, Storage and Disposal*
USA	United States of America
WHO	World Health Organization
WT	Wild type

Chapter 1 Risk assessment context

Section 1 Background

1. An application has been made under the *Gene Technology Act 2000* (the Act) for Dealings involving the Intentional Release (DIR) of genetically modified organisms (GMOs) into the Australian environment.

2. The Act and the Gene Technology Regulations 2001 (the Regulations), together with corresponding State and Territory legislation, comprise Australia's national regulatory system for gene technology. Its objective is to protect the health and safety of people, and to protect the environment, by identifying risks posed by or as a result of gene technology, and by managing those risks through regulating certain dealings with GMOs.

3. Section 50 of the Act requires that the Gene Technology Regulator (the Regulator) must prepare a Risk Assessment and Risk Management Plan (RARMP) in response to an application for release of GMOs into the Australian environment. Sections 50, 50A and 51 of the Act and sections 9 and 10 of the Regulations outline the matters which the Regulator must take into account and who must be consulted when preparing the RARMP.

4. The *Risk Analysis Framework* (RAF) (OGTR, 2013) explains the Regulator's approach to the preparation of RARMPs in accordance with the Act and the Regulations. The Regulator has also developed operational policies and guidelines that are relevant to DIR licences. These documents are available from the Office of the Gene Technology Regulator (OGTR website).

5. Figure 1 shows the information that is considered, within the regulatory framework above, in establishing the risk assessment context. This information is specific for each application. Risks to the health and safety of people or the environment posed by the proposed supply are assessed within this context. Chapter 1 describes the risk assessment context for this application.

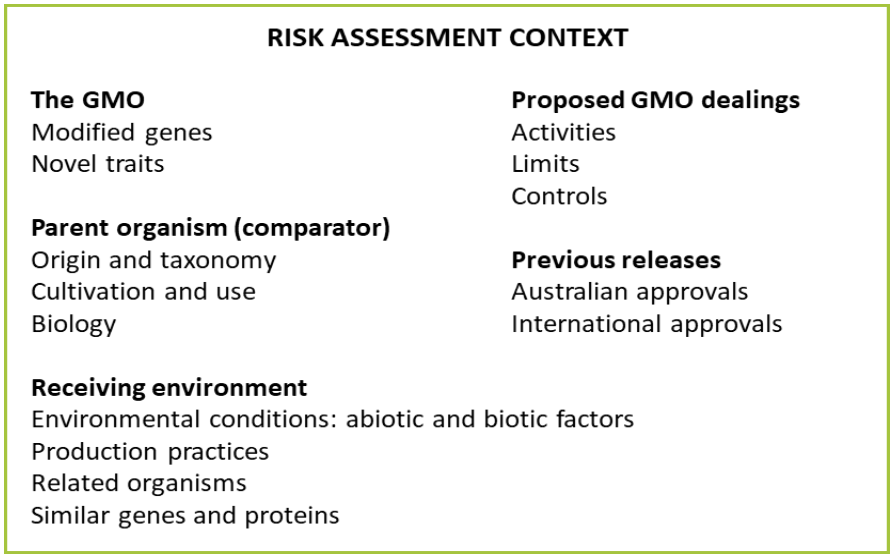

Figure 1. Summary of parameters used to establish the risk assessment context, within the legislative requirements, operational policies and guidelines of the OGTR and the RAF.

6. In accordance with Section 50A of the Act, this application is considered to be a limited and controlled release application, as the Regulator was satisfied that it meets the criteria prescribed by the Act. Therefore, the Regulator was not required to consult with prescribed experts, agencies and authorities before preparation of the RARMP.

1.1 Interface with other regulatory schemes

7. Gene technology legislation operates in conjunction with other regulatory schemes in Australia. The GMOs and any proposed dealings conducted under a licence issued by the Regulator may also be subject to

regulation by other Australian government agencies that regulate GMOs or GM products, including Food Standards Australia New Zealand (FSANZ), the Australian Pesticides and Veterinary Medicines Authority (APVMA), the Therapeutic Goods Administration (TGA), the Australian Industrial Chemicals Introduction Scheme (AICIS) and the Department of Agriculture, Fisheries and Forestry (DAFF).

8. Medicines and other therapeutic goods for use in Australia are required to be assessed for quality, safety and efficacy under the *Therapeutic Goods Act 1989* and must be included in the Australian Register of Therapeutic Goods. The TGA is responsible for administering the provisions of this legislation. Clinical trials of therapeutic products that are experimental and under development, prior to a full evaluation and assessment, are also regulated by the TGA through the Clinical Trial Approval (CTA) scheme or the Clinical Trial Notification (CTN) scheme.

9. For clinical trials, the TGA has regulatory responsibility for the supply of unapproved therapeutic products. In terms of risk to individuals participating in a clinical trial, the TGA (as the primary regulatory agency), the trial sponsor, the investigators and the Human Research Ethics Committee (HREC) at each trial site all have roles in ensuring participants' safety under the *Therapeutic Goods Act 1989*. However, where the trial involves a GMO, authorisation is also required under gene technology legislation. To avoid duplication of regulatory oversight, and as risks to trial participants are addressed through the above mechanisms, the Regulator's focus is on assessing risks posed to people other than those participating in the clinical trial, and to the environment. This includes risks to people preparing and administering the GM bacteria, and risks associated with import, transport and disposal of the GMO.

10. The International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use – Guidelines for Good Clinical Practice (ICH-GCP) is an international ethical and scientific quality standard for designing, conducting, recording and reporting trials that involve the participation of human subjects (ICH 1996). The guideline was developed with consideration of the current good clinical practices of the European Union (EU), Japan, and the United States of America (USA), as well as those of Australia, Canada, the Nordic countries and the World Health Organization (WHO). The TGA has adopted the ICH-GCP in principle as Note for Guidance on Good Clinical Practice (designated CPMP/ICH/135/95) (Therapeutic Goods Administration 2000), which provides overarching guidance for conducting clinical trials in Australia which fall under TGA regulation.

11. The National Health and Medical Research Council (NHMRC) has issued the *National Statement on Ethical Conduct in Human Research* (National Health and Medical Research Council et al., 2018). This document sets the Australian standard against which all research involving humans is reviewed. The *Therapeutic Goods Act 1989* requires that the use of a therapeutic good in a clinical trial must be in accordance with the ethical standards set out in this document.

12. Approval by a Human Research Ethics Committee (HREC) is also a fundamental requirement of a clinical trial. HRECs conduct both ethical and scientific assessment of the proposal and in addition often consider issues of research governance. Other elements of governance of clinical trials that are considered by HRECs include appropriate informed consent, specific inclusion and exclusion criteria, data monitoring and GMO accounting and reconciliation.

13. DAFF administers Australian biosecurity conditions for the importation of biological products under the *Biosecurity Act 2015*. Biological products include animal or microbial derived products such as foods, therapeutics, laboratory materials and vaccines (including GMOs).

14. Analysis of biological samples collected from trial participants administered with the GMO would occur at clinical trial sites, or at pathology laboratories. These facilities are regulated by State and Territory governments and adhere to professional standards for safety, disease control (Australian Guidelines for the Prevention and Control of Infection in Healthcare (2019) and handling of pathology samples (National Pathology Accreditation Advisory Council; NPAAC).

15. The NPAAC advises Commonwealth, State and Territory health ministers on matters relating to the accreditation of pathology laboratories. NPAAC plays a key role in ensuring the quality of Australian pathology services and is responsible for the development and maintenance of standards and guidelines for pathology practices. The standards include safety precautions to protect the safety of workers from

exposure to infectious microorganisms in pathology laboratories. While compliance with NPAAC standards and guidelines is not mandatory, there is a strong motivation for pathology services to comply, as Medicare benefits are only payable for pathology services if conducted in an appropriate Accredited Pathology Laboratory (APL) category, by an Approved Pathology Practitioner (APP) employed by an Approved Pathology Authority (APA). Accreditation of pathology services is overseen by Services Australia (formerly Department of Human Services), and currently, the only endorsed assessing body for pathology accreditation is the National Association of Testing Authorities (NATA).

16. The state and territory governments regulate hospitals and other medical facilities in Australia. All public and private hospitals and day procedure services need to be accredited to the National Safety and Quality Health Service (NSQHS) Standards developed by the Australian Commission on Safety and Quality in Healthcare (the Commission) and endorsed by the state and territory Health Ministers. The Commission coordinates accreditation processes via the Australian Health Service Safety and Quality Accreditation (AHSSQA) scheme. The NSQHS Standards provide a quality assurance mechanism that tests whether relevant systems are in place to ensure that the minimum standards of safety and quality are met. The safety aspects addressed by the NSQHS Standards include the safe use of sharps, disinfection, sterilisation and appropriate handling of potentially infectious substances. Additionally, the Commission has developed the National Model Clinical Guidance Framework, which is based on, and builds on NSQHS Standards to ensure that clinical governance systems are implemented effectively and to support better care for patients and consumers.

17. Hospitals and pathology laboratories, including their workers, managers and executives, all have a role in making the workplace safe and managing the risks associated with handling potentially infectious substances including the proposed GMO. There are minimum infection prevention practices that apply to all health care in any setting where health care is provided. These prevention practices were initially developed by the Centers for Disease Control and Prevention (CDC) and are known as the standard precautions for working with potentially infectious material. The standard precautions are described in the Australian Guidelines for the Prevention and Control of Infection in Healthcare (2019).

Section 2 The proposed dealings

18. Melius is seeking authorisation to carry out a Phase 1 clinical trial to assess the safety and efficacy of a genetically modified (GM) *E. coli* that is modified to have a survival advantage in an inflammatory environment and a reduced survivability in the environment.

19. The dealings involved in the proposed clinical trial are:

(a) import the GMO;

(b) conduct the following experiments with the GMO:

i. prepare the GMO for administration to trial participants;

ii. administer the GMO to clinical trial participants by oral ingestion;

iii. collect samples from trial participants;

iv. analyse the samples;

(c) transport the GMO;

(d) dispose of the GMO;

and the possession (including storage), supply and use of the GMO for the purposes of, or in the course of, any of these dealings.

2.1 The proposed limits of the trial

20. The clinical trial is proposed to take place over a five-year period from the date of issue of the licence. Up to 36 participants in Australia would receive one to four doses of the GMO or a placebo via oral ingestion.

21. The clinical trial would take place at Mater Misericordiae Hospital Brisbane. Additional sites in Brisbane may be engaged for the recruitment of healthy volunteers if needed, but this is not anticipated by the applicant.

2.2 The proposed controls to restrict the spread and persistence of the GMO in the environment

22. The applicant has proposed several controls to minimise exposure to the GMO, and to restrict the spread and persistence of the GMO in the environment. These include:

- Only trained personnel would conduct dealings with the GMO.
- Staff preparing the GMO would be required to wear appropriate PPE (e.g. gown and gloves) during the procedures.
- Although the GMO would be self-administered orally, staff present would be required to also wear appropriate PPE (e.g. gloves).
- Transport to and storage of the GMO at a clinical trial facility where it would be administered would be in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* (TSDs).
- Disinfecting surfaces and equipment that come into contact with the GMO using an effective disinfectant (including, but not limited to, bleach 1000-5000 ppm; 70% ethanol or 1% Virkon™).

2.3 Details of the proposed dealings

2.3.1 Manufacturing of the GMO

23. The GMO would be manufactured in Canada and imported into Australia. The doses in the form of microbeads would be either packaged into blister packs, or in plastic vials or bottles composed of high-density polyethylene or equivalent materials. The applicant has stated that the type of primary packaging is still being finalised, and they would inform the OGTR when it has been finalised. The primary containers would then be enclosed within a secondary container of individual cartons with tamper-evident seals.

2.3.2 Transport and storage of the GMO

24. The GMO would be imported according to the packaging and labelling requirements of the International Air Transport Association (IATA) code UN3245.

25. Transport of the GMO from the Australian border would be directly to Mater Misericordiae Hospital Brisbane or other clinical trial sites if needed. The GMO would be packaged into blister packs, sealed plastic vials or bottles composed of high-density polyethylene or equivalent material. They would then be placed into a container, which would be enclosed within secondary packaging, consisting of cartons with tamper-evident seals.

26. Procedures would be in place to ensure that all transported GMOs can be accounted for, and that a loss of GMOs during transport can be detected; and access to the GMOs would be restricted to authorised persons conducting dealings under the licence, who have been informed by the licence holder of any licence conditions that apply to them.

27. The proposed method of supply and storage of the GMO, as advised by the applicant, would be in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* (TSD).

2.3.3 Clinical trial sites

28. The clinical trial would be carried out at a clinical trial site at the Mater Misericordiae Hospital, Brisbane. The applicant has also proposed that additional clinical trial sites in the Brisbane area may be used to recruit healthy volunteers if needed. Clinical trial sites would be assessed by the applicant for their ability to comply with local biosafety requirements. Clinical trial sites will need to meet the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good

Clinical Practise (GCP) guidelines (ICH Guideline for Good Clinical Practice). Sites will also be selected based on their ability to comply with the TSDs and the licence conditions.

2.3.4 The clinical trial

29. The applicant proposes a Phase 1 study, which is to be conducted at clinical trial sites at the Mater Misericordiae Hospital, Brisbane, or other clinical trial sites in the Brisbane area (as noted in Section 2.3.3). The study aims to assess the safety and tolerability of the GMO.

30. The study would involve healthy participants and patients with ulcerative colitis who will receive either one dose or four oral weekly doses of the GMO or receive a placebo. All participants would be monitored for up to 28 days after final dose.

31. The study is proposed to occur in 2 stages, the first in healthy participants, before administration to patients with mild to moderate ulcerative colitis. All participants (healthy and patients with ulcerative colitis) would be enrolled in 3 cohorts of escalating doses (10^6 colony forming units (CFU); 10^7-10^8 CFU; and 10^9 CFU), with a placebo control.

2.3.5 Selection of trial participants

32. Inclusion criteria proposed by the applicant relevant to this assessment include that trial participants must:

- Be 18 to 75 years of age.
- Agree to use effective barrier contraceptives and abstain from unprotected anal sex for the duration of the trial.

33. Relevant exclusion criteria include participants who:

- Have diagnosis of any non-Inflammatory bowel disease related diarrhoeal illness (e.g. *Clostridioides difficile*, coeliac disease or parasitic infections) within 3 months prior to randomisation.
- Use of probiotics within the 2 weeks prior to randomisation.
- Use of agents that alter gut transit time including laxatives, anti-diarrhoeal medications and diabetic or weight loss medications.
- Receipt of faecal microbiota transplantation (FMT) or other faecal-derived preparation within 6 months prior to randomisation.
- Use of antibiotics.
- Have previously had colectomy, ostomy, or other intestinal surgery (excluding cholecystectomy or appendicectomy).

34. In addition, participants may be excluded for any reason that, in the opinion of the investigator, makes the participant unsuitable for the study.

2.3.6 Preparation of the GMO for administration

35. The GMO would be in a microencapsulated form, as a bead. The doses of the GMO for administration would be prepared for dispensing as a bead in research pharmacies within the hospital or clinical trial sites by trained personnel. Access to the GMO during preparation would be restricted to the pharmacy personnel. Training would be provided by the licence holder in line with the licence conditions.

2.3.7 Oral administration of the GMO

36. The GMO would be self-administered orally (one bead) with water at clinical trial sites in the presence of a medical professional wearing gloves. Participants would then be instructed to wash their hands with soap and water after handling the bead. Administration of all doses would occur in the clinical trial sites.

2.3.8 Decontamination and disposal of the GMO

37. The applicant has stated that all decontamination of surfaces and spill management procedures would be conducted in accordance with the clinical trial site guidelines, OGTR requirements and biosafety training that would be provided to all personnel involved in dealings with the GMO.

38. Surface decontamination is proposed to occur before and after GMO handling and at the end of each working day with commonly used disinfectants at the appropriate contact time (e.g. bleach 1000-5000 ppm; 70% ethanol and 1% Virkon™).

39. Any accidental spills or shedding of the GMO (e.g. rupture of samples containing the GMO, contaminated faeces or vomit,) is proposed to be immediately contained. The area would be isolated, and the material would be covered with absorbent material, followed by liberal application of disinfectants over the material and surrounding area for an appropriate minimum contact time for decontamination. Personnel cleaning up the area would be wearing the appropriate PPE (gloves, gowns, masks, and eye protection). Spill kits containing PPE, absorbent material and disinfectants would be maintained at all sites handling the GMOs and any incidents would be recorded and reviewed in accordance with the site's standard operating procedures and the licence conditions.

40. Any unused doses of the GMO would be disposed of in accordance with the clinical trial site biological waste guidelines and the TSDs at the end of the trial.

41. Participants would also be given instructions to wash their hands with soap and water following toilet use.

2.3.9 Sample collection and analysis

42. Samples would be collected at specified intervals for the duration of the clinical trial. Details of the types of samples and collection times have been declared commercial confidential information (CCI).

43. Samples would be collected at the clinic, and some sampling (not involving sharps) may also be carried out at home. Participants would be provided airtight containers and plastic sealable bags for home collected samples; and instructions on proper hand hygiene practices and appropriate storage of home collected samples. All contaminated waste from home-collected samples would also be double bagged and returned to the clinical trial site for decontamination.

44. Analysis of samples that may contain GMOs would occur in independent pathology laboratories.

2.3.10 Personal protective clothing

45. Clinical trial staff involved in the preparation and administration of the GMO to trial participants would wear gloves and lab coats.

46. In the clean-up of spills or shedding of the GMO (e.g. vomit, diarrhoea), the clinical trial staff would wear gloves, gowns, masks and eye protection.

2.3.11 Training

47. The applicant has indicated that staff handling the GMO during preparation and administration would be experienced research pharmacy personnel and medical professionals, respectively.

48. If the licence is issued, the applicant has stated that they would be responsible for ensuring all personnel handling the GMO would be trained in the licence conditions.

2.3.12 Accountability and Monitoring

49. The applicant has stated that procedures would be in place to ensure that all stored GMOs can be accounted for, and any loss of the GMO can be detected.

50. Participants would be monitored for any adverse events following the administration of the GMO and during the follow up visits to the clinical trial sites.

2.3.13 Contingency plans

51. In the event of unintentional release of the GMO due to spills or shedding of the GMO, personnel would be instructed to follow spill management procedures, including that;

(a) the GMO will be contained to prevent further dispersal;

(b) persons cleaning up the GMO will wear PPE including gloves, gown, masks and eye protection;

(c) the exposed area will be decontaminated with an appropriate chemical disinfectant effective against the GMO;

(d) any material used to clean up the spill or PPE worn during the clean up will be decontaminated;

(e) clinical trial staff will notify the licence holder as soon as reasonably practicable; and

(f) the licence holder will notify the Regulator as soon as reasonably practicable.

52. In the event of exposure of people to the GMO via inhalation, direct contact with facial mucosa, or ingestion, the applicant proposes such persons would be instructed to:

(a) rinse their eyes, nose, and mouth thoroughly with water;

(b) monitor for any gastrointestinal discomfort; and

(c) report the incident to the licence holder, the institution's IBC and the trial sponsor.

Section 3 Parent organism

53. The GMO is derived from the bacterium *Escherichia coli* Nissle 1917 strain. It is a member of the genus *Escherichia* in the family and the family Enterobacteriaceae. It meets the criteria to be classified as a Risk Group 1 organism in accordance with the Australian/New Zealand Standard 2243.3:2022 (Standards Australia/New Zealand, 2022). The characteristics of the parent organism provide a baseline for comparing the potential for harm from dealings with GMOs. As such, the relevant biological properties of *E. coli* Nissle strain will be discussed here.

54. The classification *Escherichia* has been complex, but it is mainly classified to 7 species (*E. coli*, *E. albertii*, *E. fergusonii*, *E. hermannii*, *E. marmotae*, *E. ruysiae* and E. *whittamii*) (Cobo-Simón et al., 2023; Meier-Kolthoff et al., 2014). The species *E. coli* is further characterised into fourteen phylogenetic groups (A, B1, B2-1, B2-2, C, D1, D2, D3, E1, E2, F, G, Shig1 and Shig2), which includes the genus of *Shigella* as it has been shown to be a subspecies of *E. coli* (Abram et al., 2021).

55. *E. coli* was first described by Theodor Escherich in 1885 (Lim et al., 2010). *E. coli* are facultative anaerobic, gram negative, non-sporulating rod shaped bacteria. Facultative anaerobes can survive both in aerobic as well as in anaerobic conditions. *E. coli* can be either non-motile or motile, with a flagellum, and grow best at 37°C. *E. coli* can either live inside a host or in the environment. Inside a host, *E. coli* can either be commensal or pathogenic.

3.1 Commensal *E. coli*

56. *E. coli* usually has a commensal relationship with the host, deriving a steady supply of nutrients as well as protection and dissemination from the host. This interaction, however, provides some benefits for the host as *E. coli* microbiota prevents colonisation by and growth of pathogens, by producing bacteriocins and other mechanisms (Hudault et al., 2001; Rastegarlari et al., 1990; Schamberger et al., 2004; Vollaard and Clasener, 1994). *E. coli* has a wide host range, colonising mammals, birds, reptiles and amphibians (Berg et al., 1983).

57. Commensal *E. coli* strains are found in the large intestine, especially in the caecum and the colon, mainly in the mucus layer covering the epithelial cells throughout the tract. They are shed into the intestinal lumen with degraded mucus components and are excreted in the faeces. It is estimated that there are 10^7-10^9 *E. coli* in each gram of human faeces (Tenaillon et al., 2010). *E. coli* has adapted to its ecological niche and competes with other bacteria for nutrients (Licht et al., 1999; Poulsen et al., 1994; Rang et al., 1999).

3.2 Pathogenic *E. coli*

58. Although most strains of *E. coli* are non-pathogenic and are commensal residents of the human gut (Gordon and Cowling, 2003), some can cause diseases. *E. coli* is estimated to cause hundreds of thousands of deaths a year (Russo and Johnson, 2003). Pathogenic *E. coli* have virulence factors that are not present in commensal *E. coli*, such as toxins, adhesins, protective coats and invasins.

59. Pathogenic *E. coli* strains causing infection within the gut can be classified based on the symptoms they cause such as enteropathogenic *E. coli* (EPEC), enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC) and enteroaggregative *E. coli* (EAEC) (Vila et al., 2016). Most commonly, infections with these *E. coli* cause diarrhoea or gastroenteritis and are often acquired though eating contaminated food. Some EHEC have a virulence factor that leads to the production of a toxin called shiga, so they are also called shiga toxin-producing *E. coli* (STEC). STEC infections can cause bloody diarrhoea, abdominal cramps, vomiting and sometimes a serious condition called haemolytic uraemic syndrome which can be fatal (Lim et al., 2010). Large outbreaks of STEC sometimes occur in developed countries but are relatively uncommon in Australia. 822 STEC infections were notified in Australia between 2000 and 2010 along with 169 cases of haemolytic uraemic syndrome (Vally et al., 2012). Antibiotics are not recommended for STEC infections and may be harmful (2018).

60. Pathogenic *E. coli* can sometimes cause disease outside of the gut and are therefore called extraintestinal pathogenic *E. coli* (ExPEC). These *E. coli* have often colonised the human gut without causing issues but become a problem when they are able to spread to other body sites. Some of the same virulence factors (such as P fimbriae and specific capsules) that make these *E. coli* damaging when they are outside of the gut, help them to successfully colonise the human gut (Vila et al., 2016).

61. ExPEC are the most common cause of urinary tract infections (UTIs) and are sometimes called urinary pathogenic *E. coli* (UPEC). UTIs include infection of the bladder, urethra, ureters and kidneys. They are normally treated with antibiotics, but if left untreated (or if the antibiotics used are ineffective) can lead to serious complications (healthdirect, 2020). *E. coli* that cause UTIs have multiple virulence factors; adhesins that help them stick to cells, toxins that help them spread into tissues and evade the immune system, the ability to form biofilms, and iron acquisition mechanisms that help them get nutrients (Vila et al., 2016).

62. A large study into skin and soft tissue infections found *E. coli* was the third most common cause of infection (*Staphylococcus aureus* was the most common cause) (Moet et al., 2007). When these infections are not self-limiting, they are treated with antibiotics to ensure the infection does not spread or enter the bloodstream.

63. Under certain conditions including after surgical operations or immunosuppression, previously commensal *E. coli* can act pathogenically. Bloodstream infections are the most common and life-threating complication after solid organ transplants, and about 37% of these are caused by *E. coli (AAP, 2018)*.

3.3 Free-living *E. coli*

64. It is estimated that half of the *E. coli* population resides in water and sediments (Savageau, 1983). The oral – faecal route is the main mode of transmission and distribution of *E. coli* and its presence in water is often used as an indicator of faecal pollution (Russell and Jarvis, 2001; Savageau, 1983). However, more recent reports show that some *E. coli* are naturalised to soil, sand and sediments (Jang et al., 2017).

3.4 *E. coli* Nissle strain

65. The parent organism *E. coli* Nissle strain (EcN) was first isolated by Alfred Nissle in 1917 and is a commensal and non-pathogenic strain of *E. coli* that belongs to the B2 phylogenetic group (Sonnenborn, 2016; Wassenaar, 2016). Although the B2 phylogenetic group is typically associated with *E. coli* strains that can cause disease, unlike pathogenic strains, EcN does not express disease-causing factors and is not known to contain any conjugative plasmids or antibiotic resistant genes (Grozdanov et al., 2004; Nowrouzian et al., 2005). It is reported to contain 2 cryptic plasmids pMUT1 and pMUT2 that have unknown functions, but have been used as a detection method for EcN (Blum-Oehler et al., 2003). Exposure to human blood serum can kill EcN, and hence it is easily cleared by the immune system (Grozdanov et al., 2002).

66. EcN is the most frequently used probiotic *E. coli* strain. It is commercially available as an over-the-counter probiotic in capsules and is mostly used to treat inflammatory bowel disease. In Australia, EcN (Mutaflor®) is a registered complementary medicine with the TGA. The maximum recommended daily dose is 10^{11} CFU, and treatment is usually well tolerated and does not cause significant changes to stools in healthy people but can reduce constipation. Some ingested bacteria pass through the gut rapidly, whereas bacteria that live in the gut for a significant amount of time are considered to have colonised the gut. A systematic review of multiple studies using the EcN strain suggests that it is not very efficient at colonising the adult human gut long term (Wassenaar, 2016). Another review has also reported that the EcN can maintain its colonisation of healthy adult mice and humans for up to 24 weeks after the last treatment, but the majority of individuals clear EcN in 2 weeks (Gurbatri et al., 2024). However, other reports claim it is a good coloniser (Lasaro et al., 2009; Lodinová-Zádniková and Sonnenborn, 1997).

67. EcN has also been used in studies for treatment of diarrhoea and urinary tract infections in dogs, pigs, calves; as a probiotic against other pathogenic bacteria in chicks; and to improve the immunity and egg laying performance of Japanese quails. No serious adverse events or toxicity were identified in these animals (Helmy Yosra et al., 2022; Mourand et al., 2021; Rudinsky et al., 2023; Sedaghat et al., 2025; von Buenau et al., 2005; Wu et al., 2023).

68. EcN has been shown to directly inhibit the growth of various pathogenic bacteria by the secretion of various antimicrobial molecules and enzymes (e.g. *Escherichia spp., Salmonella spp., Klebsiella spp., Shigella spp., Pseudomonas spp., Staphylococcus spp.*). It can also prevent biofilm formation by other EHEC pathogens, *Salmonella enterica*, *P. aeruginosa, Staphylococcus aureus* and *S. epidermidis*. Bacteria that form biofilms are highly tolerant of external stresses such as antibacterial agents and are the major cause of chronic and medical device related infections (Chen et al., 2023; Fang et al., 2018; Sassone-Corsi et al., 2016; Sonnenborn, 2016).

69. EcN has anti-inflammatory effects in the intestinal epithelial cells (IEC) in inflammatory bowel disease (IBD). A review by Chen et. al., has suggested that EcN does this by competing against harmful bacteria for resources; stimulating IECs to produce various molecules to resist pathogenic bacteria and inhibit the expression of pro-inflammatory molecules; and stimulating IECs to repair the intestinal epithelial barrier (Chen et al., 2023).

70. It has recently been reported that *E. coli* belonging to the B2 phylogenetic group (including EcN), contains polyketide synthase (*pks*) islands that encode colibactin, a genotoxin (Auvray et al., 2021). The *E. coli pks* island is encoded by 19 genes (*clbA* – *clbS*) (Auvray et al., 2021). Colibactin is reported to induce double stranded breaks and chromosomal mutations (Falzone et al., 2024). Some *in vitro* and *in vivo* laboratory studies have demonstrated that EcN is able to cause mutations (Pleguezuelos-Manzano et al., 2020; Rosendahl Huber et al., 2024). Studies have also shown that the deletion of *clbA* gene from EcN, is able abrogate its ability to cause DNA damage. However, this deletion impairs its probiotic activity (Olier et al., 2012). Conversely, other studies have demonstrated that EcN does not cause/induce genotoxicity (Dubbert et al., 2020; Janosch et al., 2019). No information was found that shows that EcN can cause cancer in humans.

3.5 Genetics of *E. coli*

71. The genome size varies widely across *E. coli* strains, with the average genome containing around 5000 genes. Only 1700 genes are conserved among all strains (these are commonly referred to as ‘strict core’) and 3000 genes are conserved in at least 95% of the strains (commonly referred to as ‘soft core’) (Kaas et al., 2012). Hence each strain contains genes from the core genome and genes from an extended pool of approximately 8000 genes. This provides a high level of plasticity in the genome and also reflects the adaptive nature of the organism (Tenaillon et al., 2010).

72. The NCBI RefSeq and NCBI databases predict that EcN has 5126 and 5409 genes respectively (National Centre for Biotechnology Information, 2025). As mentioned in Section 3.4, EcN is not known to have any native conjugative plasmids or genes that carry resistance to antibiotics.

3.5.1 Horizontal gene transfer (HGT)

73. In addition to a large gene pool, *E. coli* can exchange genetic elements with other bacteria present in the surrounding environment. Genetic elements are thought to move horizontally (to compatible bacteria) and vertically (to offspring) as they can help bacteria adapt to changing environments (Kaper et al., 1995) and contribute to the development of novel strains and pathotypes.

74. *E. coli* carry genetic material in chromosomes and plasmids. Chromosomes contain the essential genetic material of *E. coli* and are generally vertically inherited by the offspring from the parent. Plasmids are usually smaller packets of DNA that exist separately from the bacterial chromosomes and can replicate independently of chromosomes. Enterobacterales, which include *E. coli*, often carry multiple plasmids simultaneously (Dionisio et al., 2019; Garcia et al., 2007).

75. There are four main genetic mechanisms that enable the horizontal transfer of genetic elements in *E. coli*: conjugation, transformation, transposition and transduction (See Figure 3).

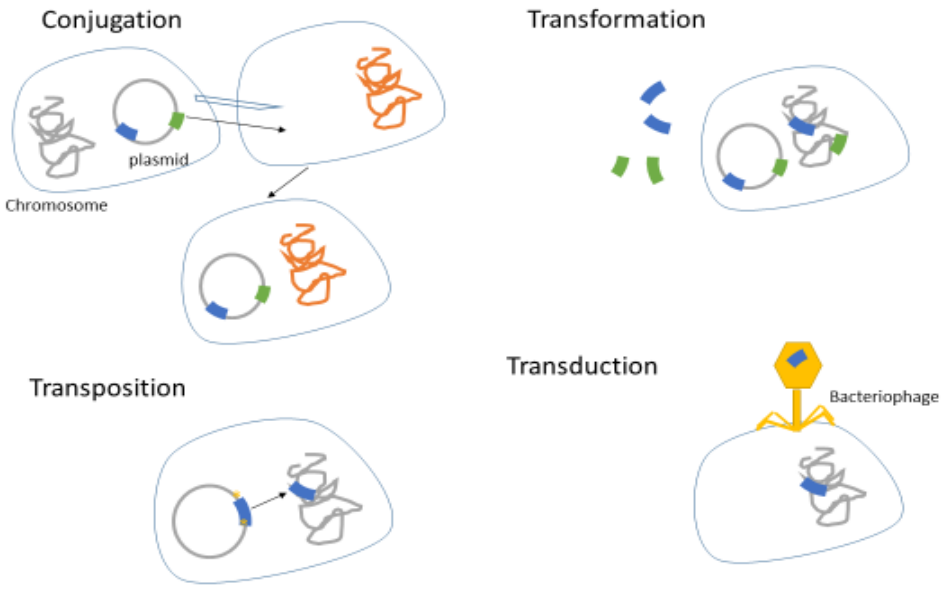

Figure 3. Common mechanisms of horizontal gene transfer in bacteria

76. **Conjugation** describes the direct transfer of DNA from one bacteria to another and is arguably the most important mechanism of horizontal gene transfer in bacteria (Norman et al., 2009; Sørensen et al., 2005). It involves 2 bacteria coming into physical contact and forming a mating pair. The donor bacterium produces a filamentous pilus that allows a copy of the plasmid to travel across into the recipient bacterium Both the donor and acceptor now have a copy of the plasmid.

77. **Transformation** in *E. coli* involves the induction of competence, DNA binding followed by fragmentation of the DNA, uptake and stable maintenance of the DNA by either integration in the genome (recombination) or recircularisation of plasmid DNA (Harrison and Brockhurst, 2012; Sørensen et al., 2005).

78. **Transduction** is the movement of genetic material with the help of bacteriophages. Erroneously packed host DNA can be transferred to other bacteria upon its infection with the phage. In theory, any region of the bacterial genome can be transferred in that way, including plasmids, but the DNA will not be retained by the host unless the phage integrates into the bacterial genome (prophage). The regions co-integrated with prophage DNA are commonly the flanking regions of the prophage insertion site (Berg et al., 1983).

79. **Transposition** describes the translocation of a discrete segment of DNA (the transposable element or transposon) from a donor site to non-homologous target sites. Transposable elements encode the machinery required to execute such rearrangements in addition to other determinants such as antibiotic resistance genes and genes for virulence factors. In general, transposition is an infrequent event probably because of its capacity for deleterious effects in the host. Usually, a transposon is translocated onto a

plasmid upon conjugation. This may be followed by the integration of the transposon into the chromosome. For many transposons, however, plasmids rather than the bacterial chromosome appear to be the preferred target (Craig, 2014).

80. Laboratory studies have shown that HGT can occur in EcN both *in vitro* and *in vivo* (Zebrafish and mice) (Fang et al., 2024; Frazão et al., 2019).

3.6 Bio-distribution and shedding

81. The principal route by which the GM bacteria may enter the wider environment following inoculation is via shedding. Further, GM bacteria could also enter the environment via accidental spills of unused GMO preparation.

82. Human faeces is estimated to contain about 10^{12} bacteria per gram (Sender et al., 2016) and healthy adults produce in the order of 100 g of faeces per day in western countries (Cummings et al., 1992). So approximately 10^{14} bacteria per person per day may enter sewage. Around 90% of these bacteria will belong to the Firmicutes (also called Bacillota) and Bacteroidetes (also called Bacteroidota) phyla (Rinninella et al., 2019). It has been estimated that there are about 10^{8} CFU of *E.coli* per gram of faeces (Zuo et al., 2011) or 10^{7}-10^{9} *E.coli g/faeces* (Tenaillon et al., 2010).

83. Human gut microbiota is excreted into sewage and wastewater, where it undergoes standard waste treatment processes (which can vary significantly), prior to the water being released back into the environment. Sewage treatment is likely to be effective at removing the GM bacteria from sewage. However, due to variable levels of sewage treatment in the wastewater plants (Toze et al., 2012), this could result in varying amounts of bacteria remaining in the sewage and could result in dispersal of some microbiota, including GM bacteria, directly into rivers or marine environments.

84. Bacterial populations in raw sewage include human faecal bacteria, bacteria resident in the sewer system infrastructure, and environmental bacteria originating from grey water and surface runoff (Shanks et al., 2013). In untreated sewage samples collected from 13 wastewater treatment plants in the United States, the most abundant bacterial phyla were Proteobacteria (also called Pseudomonadota), which includes *E. coli* (average 62%), Firmicutes (average 21%) and Bacteroidetes (average 13%) (Shanks et al., 2013). Similarly, in activated sludge samples collected from 14 wastewater treatment plants in east Asia and North America, the most abundant phyla were Proteobacteria (35-65%), Firmicutes (averaging 8%), Bacteroidetes (averaging 7%) and Actinobacteria (averaging 7%) (Zhang et al., 2012).

85. In urban areas, most wastewater is processed at centralised wastewater treatment plants (WWTPs). Processes in WWTPs vary but generally the wastewater undergoes a primary treatment process involving sedimentation, followed by a secondary treatment where aeration is used to allow bacteria to digest organic matter. Some, but not all, WWTPs use tertiary treatment to disinfect the water further via chlorination, ozonation, UV treatment or other methods. After treatment, most wastewater is returned to the ocean, a lake, or a river. A UK study of 162 WWTPs found that primary treatment did not reduce the concentration of faecal indicator bacteria much, but secondary treatment reduced faecal indicator bacteria by 95-99%, and tertiary treatments reduce this by a further 93-97% (Kay et al., 2008). Overall, this is a reduction in bacteria of up to 3000-fold.

86. In 2022-2023, approximately 58% of urban sewage in Australia underwent tertiary wastewater treatment (Bureau of Meteorology, 2024) . A small proportion of Australian urban sewage, for example in inland towns, may only undergo secondary wastewater treatment prior to effluent discharge into inland waters (Water Quality Australia Sewerage System Guidelines website, accessed 10/11/2025). Untreated sewage is sometimes released from urban sewage systems due to overflow events, particularly during wet weather. In 2022-2023, the volume of wastewater losses and spills in Australia was approximately 3.5% of total wastewater collected (Bureau of Meteorology, 2024). Some sewage overflows enter the ocean, where the GMO is not expected to survive, but other sewage overflows occur on land or enter inland waters and could release live GMO.

87. Urban sewage treated at a wastewater treatment plant produces biosolids as well as effluent. In 2023, about 85% of biosolids produced in Australia were reused, including about 79.3% that were applied

as fertiliser to agricultural land (Australian Biosolids Statistics website, accessed 10/11/2025). Roughly half of biosolids for reuse are treated to grade A level, which involves almost complete pathogen kill, and the other half are treated to grade B level, which involves a significant reduction in pathogens (Darvodelsky, 2012). Grade A biosolids would not contain live GMO, however, some GMO could survive in Grade B biosolids, which typically achieve a 1.5-2 log reduction in microorganism concentrations compared with raw sewage solids (Department of Environment and Science, 2019). In Queensland, the use of biosolids is regulated under the End of Waste (EOW) code for Biosolids (ENEW07359617) issued by the Queensland Government in accordance with section 159 of the *Waste Reduction and Recycling Act 2011*. Biosolids must meet the requirements of <100 most probable number (MPN) of *E. coli* per gram of dry weight for it to be used.

88. An analysis of raw and treated wastewater from 4 wastewater treatments plants across Australia found an average of 126 different genera of bacteria were present (Ahmed et al., 2017). The 10 most abundant genera were *Pseudomonas, Arcobacter, Bacteroides, Paludibacterium, Conchiformibius, Flavobacterium, Polynucleobacter, Acinetobacter, Parabacteroides,* and *Cloacibacterium.* A study of 4 WWTPs in Queensland found that human pathogenic *E.coli* could sometimes survive tertiary treatment and reach the environment (Anastasi et al., 2010). Determining the number of *E.coli* in the environment that came from waste water is complicated by birds and other animals carrying similar *E.coli* strains to humans (Anastasi et al., 2012).

89. Some human waste does not enter commercial wastewater treatment but is instead subject to various types of on-site treatment. These include septic systems, aerated wastewater treatment systems and dry composting toilets. Generally, these treatments are less effective at killing bacteria compared to wastewater treatment plants.

3.7 Control, environmental stability and decontamination methods

90. EcN is sensitive to any broad-spectrum antibiotics against gram-negative bacteria (Sonnenborn and Schulze, 2009). In most cases, people will recover without the use of antibiotics.

91. *E. coli* can survive in the environment (soil, manure, water) for periods ranging from weeks to a year (van Elsas et al., 2011). Persistence in the environment depends on various factors such as availability of nutrients, temperature, oxygen and pH (van Elsas et al., 2011). Conditions in the gut would be more favourable to the persistence of *E. coli* than those in the broader environment due to the larger variability of the factors that affect persistence in the broader environment (Petersen and Hubbart, 2020).

92. All bacteria can be killed by autoclaving or high-temperature incineration (Rutala et al., 2008). Ethanol (60-80%), formaldehyde (4%) and Virkon (1%) are effective disinfectants for vegetative bacteria, but they lack sporicidal action or require long contact time (2 – 20 hours for tested species) to kill bacterial spores. Hypochlorite (0.5%) kills both vegetative bacteria and spores within 10 minutes contact time but is less effective in the presence of organic matter (Russell, 1990; Rutala et al., 2008). Methods of decontamination effective against the parent organism, EcN, are expected to be equally effective against the GMO.

Section 4 The GMO - nature and effect of the genetic modification

93. The GMO is based on EcN and has been genetically modified to enable it to colonise the inflamed gut and to reduce its ability to survive in the environment. The modifications may also result in reduced ability to colonise a healthy gut. The GMO is designed to treat patients with ulcerative colitis, which is a form of inflammatory bowel disease (IBD).

94. The GMO has been modified by the insertion of the tetrathionate reductase (*ttr*) operon and the deletion of 2 genes (Gene A and Gene B – details have been declared CCI), which reduce its ability to survive outside the gut and possibly reduces its ability to colonise a healthy gut compared to WT in mouse studies. Further details of the genes are described in sections below.

4.1 Tetrathionate reductase operon

95. The tetrathionate reductase (*ttr*) operon from *Salmonella enterica* serovar Typhimurium is inserted into the region encoding Gene B of EcN to generate the GMO. The *ttr* operon encodes the structural and regulatory genes (ttrA, B, C, S and R proteins) that form the tetrathionate reductase enzyme complex, to allow bacteria in the genus *Salmonella, Proteus* and *Citrobacter* to use tetrathionate in anaerobic conditions (Hensel et al., 1999). The ability to use tetrathionate under inflammatory and anaerobic conditions provides a growth advantage in *S. enterica* compared to other gut microbiota (Winter and Bäumler, 2011).

96. Recently, the *ttr* operon has also been isolated in a novel strain of *E. coli* (Adsit et al., 2022). It was deduced that the *ttr* operon in this novel *E. coli* was likely of *Citrobacter* lineage, acquired through horizontal transfer and likely chromosomal (Adsit et al., 2022). The study also determined that the *ttr* operon is present in less than 1% of the *E. coli* genomes in the National Centre for Biotechnology Information (NCBI) database. This suggests that *ttr* operon is becoming established in the *E. coli* population (Adsit et al., 2022).

4.2 Other genes

97. As with most biological organisms, there are various critical enzymes in *E. coli* that are involved in metabolism and biosynthesis pathways, DNA replication and the generation of essential molecules for the survival of *E. coli*. The GMO contains deletion of 2 genes encoding enzymes in EcN that reduce its ability to survive outside the gut and possibly reduce its ability to colonise a healthy gut compared to WT in mouse studies. Details of these modifications have been declared as CCI. This information will be made available to the prescribed experts and agencies that will be consulted on this application.

4.3 Characterisation of the GMO

4.3.1 Genetic stability and molecular characterisation

98. The applicant has stated that the glycerol stocks of the GMO have remained stable for at least 10 years when stored at -80°C, as determined by genetic sequence analysis. The master cell bank (MCB) used to generate the working cell bank (WCB) is tested annually for genomic stability.

99. However, there have been no studies of the genetic stability of the GMO under repeat passaging conditions. A study investigating the stability of the parent EcN strain during a scale up manufacturing process (continuous passaging of 140-160 generations) demonstrated that the mutational hotspot to be within the pMUT plasmid, which has been modified to express different proteins (Munkler et al., 2024). The native cryptic plasmids pMUT1 and pMUT2 are only found in EcN and have been reported to be stable within EcN both *in vitro* and *in vivo* (Kan et al., 2021; Ou et al., 2016; Sonnenborn and Schulze, 2009). Generally, these mutations lead to the inactivation of the protein, as it is a metabolic burden to EcN (Munkler et al., 2024). The GMO does not contain any modified plasmids, and all the genetic modifications were carried out in the chromosome of EcN and hence likely to be more genetically stable.

100. EcN has also been shown to be stable through 100 serial passages *in vitro*, and in newborn children for 24 months; to not pick up plasmids that contain virulence factors (IncFI and IncFII types); and to not take up phage-encoded genetic information for the production of Shiga-like toxins (Sonnenborn and Schulze, 2009).

101. As mentioned in Section 3.5.1, horizontal gene transfer can occur via transduction through bacteriophages integrating into the bacterial chromosome via prophage attachment sites. The removal of the phage attachment site used to insert the *ttr* gene into the GMO would increase the stability of the inserted gene from further modification/integration by bacteriophage.

4.3.2 Stability in the environment and decontamination

102. The stability of this GMO in the environment (surfaces, water types and sediments) has not been tested. Methods of decontamination effective against the parent organism, EcN, are expected to be equally effective against the GMO (see Chapter 1, Section 3.7).

4.3.3 Pre-clinical studies using the GMO

103. Pre-clinical studies were carried out in healthy mice and colitis-prone mice ($Muc^{-/-}$ mice) using WT EcN and the GMO (Verdugo-Meza et al., 2024). In this pre-print publication, it was demonstrated that the GMO persists in $Muc^{-/-}$ mice for up to 22 weeks post-treatment, while the WT EcN was mostly undetectable across this time period (Verdugo-Meza et al., 2024). In contrast, the authors have indicated that healthy mice had little to no persistence of the GMO and no observable histopathological changes were observed in the colon and liver of mice receiving the GMO versus vehicle control(Verdugo-Meza et al., 2024). This suggests that the GMO had an increased ability to colonise an inflamed gut and decreased ability to colonise the healthy gut.

104. The mouse study also showed that the GMO preferentially colonises the large intestine in colitis-prone mice (Verdugo-Meza et al., 2024). Verdugo-Meza *et. al.*, also demonstrated that in an induced colitis mouse model, mice treated with the GMO had significantly lower clinical scores, lower pathology scores, and lower infiltration of macrophages and neutrophils (drivers of ulcerative colitis) compared to mice treated with WT EcN or the front-line treatment for ulcerative colitis (5-ASA) (Verdugo-Meza et al., 2024).

105. The GMO has also been tested in pigs, which share a more similar anatomy and physiology to humans. Pigs with chemically-induced ulcerative colitis treated with the GMO had increased survival rates compared to the controls (treatment with an empty microcapsule) (Verdugo-Meza et al., 2024). No GM bacteria were detected in the blood, spleen and the mesenteric lymph nodes despite the potentially compromised intestinal barrier from the induced disease (Verdugo-Meza et al., 2024).

106. There were no adverse reactions to the GMO reported in the mouse or pig studies (Verdugo-Meza et al., 2024).

4.3.4 Clinical trials using other EcN

107. As of October 2025, 16 clinical trials were listed on www.clinicaltrials.gov using EcN for treatment of conditions ranging from colon/gastric cancer; prevention of urinary tract infections; supporting therapy for diabetes; treatment of ulcerative colitis, hay fever, liver disease and irritable bowel syndrome. Outcomes of some of these studies have been reviewed by Falzone *et. al* (Falzone et al., 2024).

108. Most reported clinical trials have used an oral dose of EcN of between 1×10^9 and 25×10^9 CFU (Gurbatri et al., 2024; Kruis et al., 2012; Manzhalii et al., 2022; Petersen et al., 2014). No adverse events relating to EcN have been reported in these studies (Gurbatri et al., 2024; Kruis et al., 2012; Manzhalii et al., 2022; Petersen et al., 2014).

109. EcN enemas were investigated in a clinical trial for patients with ulcerative colitis, at doses that range from 1×10^9 to 4×10^9 viable organisms (Matthes et al., 2010). Some adverse events - gastrointestinal and thoracic disorders - were reported but most were deemed to be unrelated to the GMO (Matthes et al., 2010).

110. As mentioned in Section 3.4, the EcN parent strain (commercially known as Mutaflor®) has been used as a probiotic for over 100 years with no reported serious adverse effects in children or adults. There is one report of sepsis (bacterial infection of the blood) by EcN in a pre-term infant with very low birthweight (<1500 g) following probiotic administration (Guenther et al., 2010). However, a larger study involving 405 neonates (newborn up to 28 days after birth; >2000 g) given 10^8 CFU of EcN or a placebo control (empty capsule) showed no obvious difference in adverse events (11.7% vs 8.1%) (Olbertz et al., 2023).

Section 5 The receiving environment

111. The receiving environment forms part of the context for assessing risks associated with dealings with the GMO (OGTR, 2013). It informs the consideration of potential exposure pathways, including the likelihood of the GMO spreading or persisting outside the site of release.

5.1 Site of administration (Gastrointestinal tract)

112. The primary environment receiving the GM *E. coli* would be the gastrointestinal (GI) tract of the trial participant.

113. In a typical healthy person, whole gut transit time is estimated to be between 10-73 hours, consisting of 2-5 hours for gastric emptying, 2-6 hours to transit the small bowel and 10-59 hours for the colon (Lee et al., 2014). A meta-analysis of the effects of probiotics on intestinal transit time found that they were moderately efficacious in reducing intestinal transit time, but *E.coli* based probiotics were not included (Miller et al., 2016).

114. Antibiotic use impacts the gut microbiome. The effect depends on the class, dosage and duration of the antibiotic treatment as well factors to do with the individual patient. As well as decreasing the total number of bacteria in the gut, broad spectrum antibiotics can change the balance between bacterial species (Rinninella et al., 2019).

5.2 Presence of related bacterial species in the receiving environment

115. The presence of related bacteria may offer an opportunity for introduced genetic material to transfer between the GMO and other organisms or vice-versa in the receiving environment.

116. The human gut naturally contains a wide range of bacteria as well as archaea, viruses, phages, yeast and fungi. The human colon has been estimated to contain about 1.5 kg of bacteria (Sender et al., 2016). The composition of the human gut bacteria varies between individuals and is affected by diet, lifestyle, medical conditions and treatments, as well as geographical location. The gut microbiota is clearly involved in training the immune system, protecting against colonisation by pathogens, biosynthesising vitamins, energy generation, endocrine function and metabolising drugs and bile salts (Lynch and Pedersen, 2016). There are many other proposed interactions between the microbiome and the host.

117. In healthy adults, 90% of the gut bacteria are Firmicutes and Bacteroidetes. There are smaller amounts of Actinobacteria, Proteobacteria (including *E.coli*), and Verrucomicrobia (Rinninella et al., 2019).

118. Microbiome diversity generally increases with age. The infant gut microbiome is affected by the way they are delivered, antibiotic use and feeding patterns. Babies born vaginally have a gut microbiome similar to that around their mother's birth canal while those delivered by c-section carry bacteria similar to their mother's skin, but these differences reduce over time (Yang et al., 2016) . Additionally there are commensal bacteria in healthy human breast milk that are passed from mother to child to help the infant build a healthy microbiome (Murphy et al., 2017) . The gut microbiome of infants may be more easily persistently colonised than adult microbiomes. A study that supplemented breast-fed infants with *Bifidobacterium infantis* EV001 for 28 days found that this bacterium was still the dominant species 60 days later (Frese et al., 2017). Studies of probiotics in adults tend not to show such a dramatic and persistent effect (Zmora et al., 2018). Children are thought to develop a microbiome more similar to adults by around age three (Yang et al., 2016).

119. A large-scale study using human gut genomic data from more than 12,000 individuals, across 45 countries (Europe, North America, Asia and Africa), showed that the Enterobacteriaceae was detected in 66% of the individuals (Yin et al., 2025). Enterobacteriaceae in the human gut include several species of Citrobacter, *E. coli*, *Enterobacter cloacae, Enterobacter aerogenes, Enterobacter gergoviae, Klebsiella pneumonia, Klebsiella oxytoca, Morganelle morganii, Pantoea agglomerans, Proteus mirabilis, Serratia marcescens, Serratia plymuthica* (Chung, 2016). The large-scale genomic study determined that *Escherichia, Klebsiella* and *Enterobacter* were the most prevalent genera (Yin et al., 2025). People with ulcerative colitis have an increased population of Escherichia-Shigella (Swirkosz et al., 2023). Therefore, it is likely that participants would have bacteria of similar species present as part of their gut microbiota.

120. Although not documented, the parent EcN is likely to be present in the Australian environment as it is listed in the ARTG (TGA) as a registered complimentary medicine and commercially available for use in Australia. As mentioned in Chapter 1, Section 3.7, *E. coli* can be present in the broader environment (e.g. soil, manure, water). Therefore, it is likely that bacteria of similar species are present in the broader environment.

5.3 Presence of similar genetic material in the environment

121. The balance of a system could be perturbed by the introduction of new genetic material through horizontal gene transfer or through release of GMO into the environment. However, the effect of

perturbation would be relatively small if the genetic material was already present in the system and did not confer any selective advantage to an organism that gained this genetic material.

122. The *ttr* operon is derived from *S. enterica* serovar Typhimurium, which is already present in the Australian environment. As previously mentioned, the parent EcN would also be present in the Australian environment. As such, it is likely that organisms in the environment, both in the gut and the broader environment have been exposed to the *ttr* genes in the GMO.

Section 6 Previous authorisations

123. This GMO has not been previously authorised for clinical trials or commercial supply in any region or country. This is a first in human clinical trial.

Chapter 2 Risk assessment

Section 1 Introduction

124. The risk assessment identifies and characterises risks to the health and safety of people or to the environment from dealings with GMOs, posed by or as the result of gene technology (Figure 7). Risks are identified within the established risk assessment context (Chapter 1), taking into account current scientific and technical knowledge. A consideration of uncertainty, in particular knowledge gaps, occurs throughout the risk assessment process.

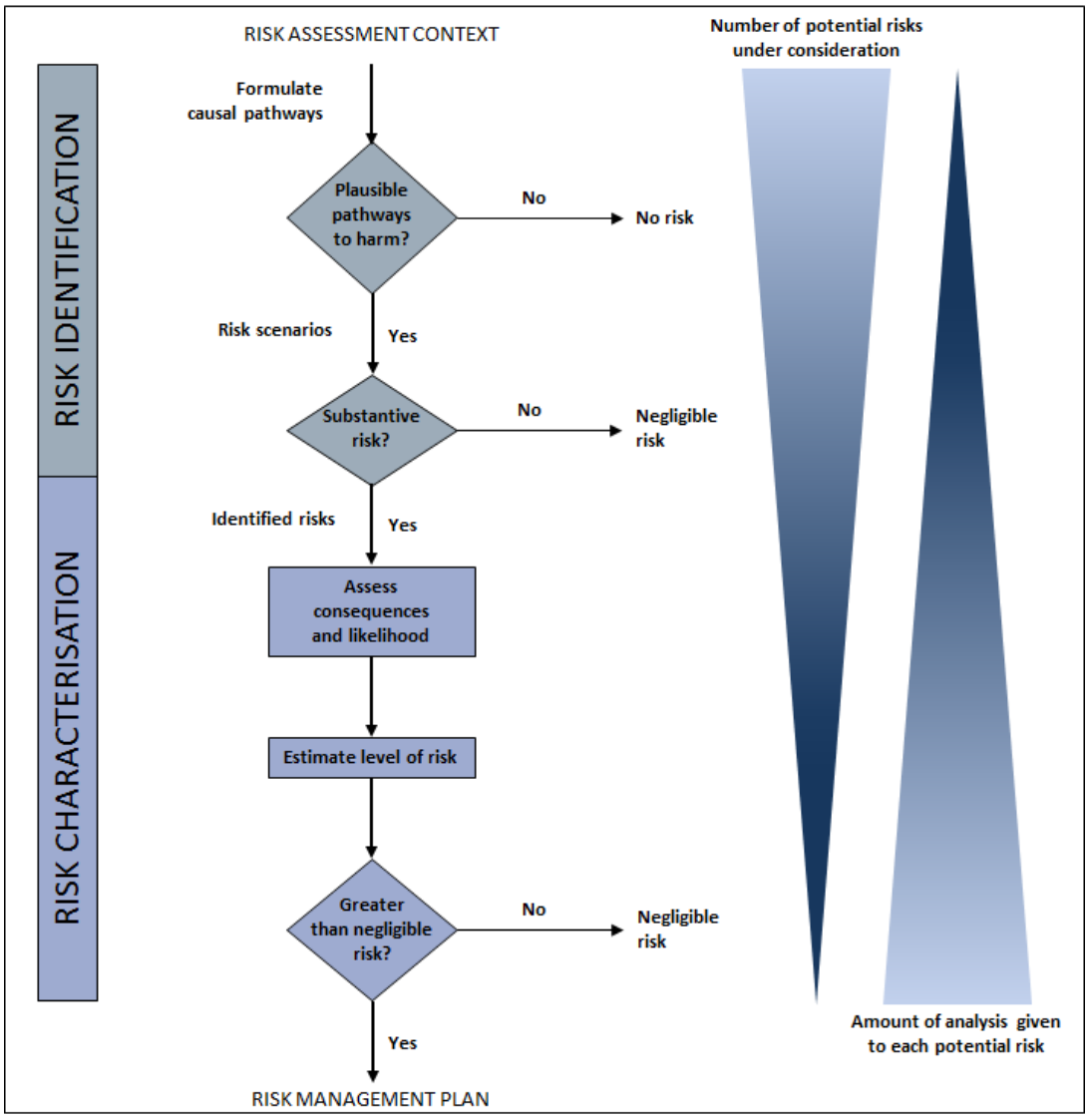

Figure 7: The risk assessment process

125. The Regulator uses a number of techniques to identify risks, including checklists, brainstorming, previous agency experience, reported international experience and consultation (OGTR, 2013).

126. Risk identification first considers a wide range of circumstances in which people, or the environment could be exposed to the GMO, or the introduced genetic material. This leads to postulating causal pathways that may give rise to harm for people or the environment from dealings with a GMO. These are called risk scenarios.

127. Risk scenarios are screened to identify substantive risks, which are risk scenarios that are considered to have some reasonable chance of causing harm. Risk scenarios that could not plausibly occur, or do not lead to harm in the short and long term, do not advance in the risk assessment process (Figure 8), i.e. the risk is considered no greater than negligible.

128. Risk scenarios identified as substantive risks are further characterised in terms of the potential seriousness of harm (Consequence assessment) and the likelihood of harm (Likelihood assessment). The consequence and likelihood assessments are combined to estimate the level of risk and determine whether risk treatment measures are required. The potential for interactions between risks is also considered.

Section 2 Risk identification

129. Postulated risk scenarios are comprised of three components (Figure 8):

i. The source of potential harm (risk source)

ii. A plausible causal linkage to potential harm (causal pathway), and

iii. Potential harm to people or the environment.

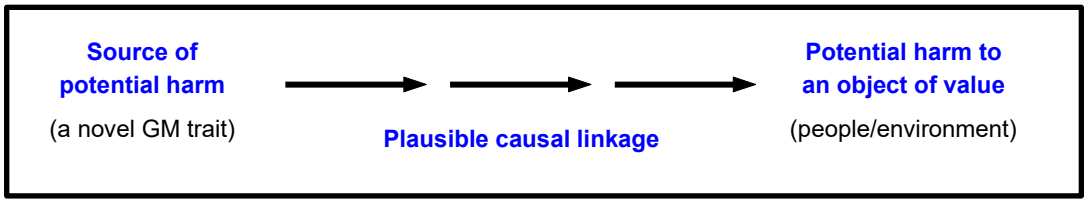

Figure 8: Components of a risk scenario

130. When postulating relevant risk scenarios, the risk context is taken into account, including the following factors detailed in Chapter 1:

- the proposed dealings
- the proposed limits including the extent and scale of the proposed dealings
- the proposed controls to limit the spread and persistence of the GMO and
- the characteristics of the parent organism(s).

2.1 Risk source

131. The parent organism is the commensal *E.coli* Nissle strain. Details of the properties of the GMO can be found in Chapter 1, Section 4. Transmission of *E. coli* is generally via the faecal-oral route and from contact with faecal material in the environment.

132. Potential sources of harm can be intended novel GM traits associated with one or more of the introduced genetic elements, or unintended effects/traits arising from the use of gene technology. Unintended effects can arise through horizontal gene transfer (HGT), the stable transfer of genetic material from one organism to another without reproduction. All genes within an organism, including those introduced by gene technology, can be transferred to another organism by HGT. A gene transferred through HGT could confer a novel trait to the recipient organism. The novel trait may result in negative, neutral or positive effects on the fitness of the recipient organism. This pathway is further considered as a potential source of risk.

133. As discussed in Chapter 1, Section 4, the GMO has been modified by the insertion of the *ttr* operon from *Salmonella enterica typhimurium* and deletions in other genes in EcN. These modified genes and their encoded proteins, or effects of deletions, are considered further as a potential source of risk.

2.2 Causal pathway

134. The following factors are taken into account when postulating plausible causal pathways to potential harm:

- the proposed dealings, which are conduct experiments (clinical trials), import, transport and disposal of the GMO and possession, supply or use (including storage) in the course of any of these dealings;
- restrictions placed on the import, transport or disposal of the GMO by other regulatory agencies, the States and Territories;
- characteristics of the parent organism;
- routes of exposure to the GMOs, the introduced gene(s) and gene product(s);
- potential effects of the introduced or deleted gene(s) and gene product(s) on the properties of the organism;
- potential exposure of other organisms to the introduced gene(s) and gene product(s) from other sources in the environment;
- potential exposure of other organisms to the GMOs in the environment;
- the release environment;
- spread and persistence of the GMOs (e.g. dispersal pathways and establishment potential);
- environmental stability of the organism (tolerance to temperature, UV irradiation and humidity);
- gene transfer by horizontal gene transfer;
- unauthorised activities; and
- practices before and after administration of the GMO.

135. As discussed in Chapter 1 Section 1.1, the TGA, the trial sponsor, the Investigators and HREC all have roles in ensuring the safety of trial participants under the *Therapeutic Goods Act 1989*, and human clinical trials must be conducted in accordance with the *National Statement on Ethical Conduct in Human Research* (National Health and Medical Research Council et al., 2018). Therefore, risk scenarios in the current assessment focus primarily on risks posed to people other than the intended GMO recipient, and to the environment.

136. The Act provides for substantial penalties for unauthorised dealings with GMOs or non-compliance with licence conditions, and also requires the Regulator to have regard to the suitability of an applicant to hold a licence prior to the issuing of the licence. These legislative provisions are considered sufficient to minimise risks from unauthorised activities. Therefore, unauthorised activities will not be considered further.

2.3 Potential harms

137. The following factors are taken into account when postulating relevant risk scenarios for this licence application:

- harm to the health of people or desirable organisms, including disease in humans or animals or adverse immune response to the GMO
- the potential for establishment of the GM *E. coli* in the environment that could cause harm to people or the environment.

2.4 Postulated risk scenarios

138. Three risk scenarios were postulated and screened to identify any substantive risks. These scenarios are summarised in Table 1 and discussed in depth in Sections 2.4.1 - 2.4.3.

139. In the context of the activities proposed by the applicant and considering both the short and long term, none of the 3 risk scenarios gave rise to any substantive risks.

Table 1 Summary of risk scenarios from dealings with the GMO

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk	Reason
1	GMO	Exposure of people (other than the trial participants) or animals (e.g. pets) via aerosols or ingestion during the: (a) Preparation and administration of the GMO (b) Shedding of the GMO (e.g. faeces, diarrhoea, vomit) (c) Import, transport, storage of the GMO (d) Disposal of the GMO ⬇ Colonisation of the GMO in the respiratory tract or gut ⬇ Infection of host cells	Ill health (e.g. diarrhoea, vomiting or gut issues) or genotoxicity	No	• The GMO has been modified to only persist in inflammatory conditions and may not persist as well as WT in a healthy gut. • The dose from accidental exposure during administration or shedding from trial participants would be low. • Only trained personnel wearing PPE would prepare, supervise the administration of and analyse the GMO. • *E.coli* Nissle strain lacks pathogenic genes. • Import would be in accordance with IATA 3245. • Transport, storage and disposal of the GMO would be in accordance with the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs.*
2	GMO	Administration of GMO to participant ⬇ Colonisation of the GMO in the participant's gut ⬇ Transfer of genetic material to or from the GMO ⬇	Ill health (e.g. diarrhoea, vomiting or gut issues) or genotoxicity	No	• The parent organism of the GMO has been safely used as a probiotic for over 100 years. • Modifications to the GMO do not change/increase its virulence. • The GMO has been modified to increase the stability of the inserted transgene. • Mutational hotspots in EcN more likely to occur in pMUT plasmid

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk	Reason
		Novel GM bacteria are shed by the participant (e.g. vomit, faeces) ⬇ Exposure of medical staff, carers or pets to novel GM bacteria shed by the participant ⬇ Colonisation of novel GM bacteria in the gut ⬇ Infection with novel GM bacteria			and not in chromosomal DNA. • Carers and participants would be advised to follow good hygiene practices in the weeks following treatment. • The dose received through accidental exposure to shedding would be far smaller than that administered. • Reversion of the GMO to the WT phenotype would not increase the pathogenicity of the microorganism above the parent strain. • Horizontal gene transfer (HGT) involving chromosomes are less efficient and do not occur in high frequences. • Exclusion criteria would further limit the presence of pathogenic bacteria for HGT to occur and cause harm.
3	GMO	Administration of GMO to participant ⬇ Colonisation of the GMO in the participant's gut ⬇ (a) no modification of the GMO (b) transfer of genetic material to or from the GMO ⬇ GMO and/or novel GM bacteria are shed by the participant ⬇	Ill health (e.g. diarrhoea, vomiting or gut issues) or genotoxicity	No	• Bacteria are substantially diluted upon entry to wastewater and 95-99% are likely to be killed by secondary wastewater treatment. This dilution makes it even more unlikely any exposed person or animal would ingest a significant amount to allow for the colonisation of the gut and develop an infection. • GMO has been modified to reduce its survivability in the environment. • EcN has been used as a probiotic with no

Risk scenario	Risk source	Causal pathway	Potential harm	Substantive risk	Reason
		GMO and/or novel GM bacteria enter wastewater system ⬇ GMO and/or novel GM bacteria survive treatment at WWTP ⬇ GMO and/or novel GM bacteria establish in the environment ⬇ A vulnerable person or animal comes into contact with the GMO and/or novel GM bacteria ⬇ GMO and/or novel GM bacteria colonises gut ⬇ Infection with GMO and/or novel GM bacteria.			reported adverse effects.

2.4.1 Risk scenario 1

Risk source	GMO
Causal pathway	Exposure people (other than the trial participants) or animals (e.g. pets) via aerosols or ingestion during the: (a) Preparation and administration of the GMO (b) Shedding of the GMO (e.g. faeces, diarrhoea, vomit) (c) Import, transport, storage of the GMO (d) Disposal of the GMO ⬇ Colonisation of the GMO in the respiratory tract or gut ⬇ Infection of host cells
Potential harm	Ill health (e.g. diarrhoea, vomiting or gut issues) or genotoxicity

Risk source

140. The source of potential harm for this postulated risk scenario is the GMO.

Causal Pathway

141. People (other than the intended trial participants) or animals (e.g. pets) could be directly or indirectly exposed to the GMO in several ways as described below. This exposure could result in colonisation of their gut and alteration of their microbiome leading to ill health (e.g. diarrhoea, vomiting or gut issues) or have genotoxic effects.

Exposure during preparation and administration of the GMO

142. There is potential for exposure of people other than the trial participant to the GMO during the preparation or administration of the GMO via direct contact of persons involved in preparation and administration of the GMO, or via incorrect dispensing of the GMO, which could lead to the wrong person receiving the GMO. This could lead to accidental contact with and/or ingestion of the GMO. The GMO is in microencapsulated form; therefore, it is very unlikely that the GMO can form aerosols during the preparation and administration.

143. As discussed in Chapter 1, Section 2.3, the preparation of the GMO would be carried out in clinical trial sites by authorised, experienced, and trained health professionals. The GMO would be self-administered by participants in the presence of trained health professionals, and they would be instructed to wash their hands with soap and water after handling the microencapsulated GMO. All personnel working in settings where healthcare is provided are required to comply with the standard precautions for working with potentially infectious material, as described in the *Australian Guidelines for the Prevention and Control of Infection in Healthcare* (2019) and the *Australian Immunisation Handbook.* Compliance with the guidelines, existing work practices and advice to trial participants would minimise the potential exposure of people to the GMOs during preparation and administration of the GMO.

144. The dose received through these pathways would be smaller than that administered during treatment. In addition, clinical trials and commercial use of the parent organism have shown no serious adverse effects at doses equivalent to the full dose of the GMO proposed for this trial. Therefore, even if an individual is inadvertently exposed to the GMO, they are unlikely to develop an adverse reaction.

145. As mentioned in Chapter 1, Section 4.2, the GMO has 2 genes deleted (Gene A and Gene B). These modifications would reduce the GMO's ability to survive outside the gut and potentially its ability to colonise a healthy gut compared to WT, based on mouse studies. Therefore, this may reduce the likelihood of establishment and persistence in a person with a healthy gut.

Exposure due to shedding of the GMO from trial participants

146. It is likely that trial participants would shed the GMO through faeces, diarrhoea, and vomiting. Vomiting is highly unlikely to occur in the healthy trial participants enrolled in this trial. However, vomiting is a symptom of ulcerative colitis, reported to occur in about 25% of patients, although this figure includes patients with severe disease (Newton et al., 2019). Caregivers, healthcare personnel and other people who are in close contact with people treated with the GMO may be inadvertently exposed to the GMO via cleaning of spills, through contact with faeces, diarrhoea or vomit, or after patient use of bathrooms. Pets could also be inadvertently exposed to the GMO from contact with faeces, diarrhoea or vomit. Caregivers, other people or pets exposed to the GMO in this way would only be expected to be exposed to low levels of the GMO. As mentioned in Chapter 1, Section 2.3, trial participants would be instructed to follow good hand hygiene practices to limit surface contamination.

147. The use of agents that can alter the gut transit time, such as laxatives, anti-diarrhoeal medications, diabetic and weight loss medications, and antibiotics, may increase the likelihood of shedding of the GMO from participants. However, participants who use these agents are excluded from the trial (Chapter 1, Section 2.3.5).

148. The GMO is also a probiotic that is intended to reduce the incidence of IBD, which would limit the occurrence of diarrhoea and vomiting.

Exposure during import, transport, and storage of the GMO

149. If the GMO was spilled during import, transport and storage, this could result in exposure to people or animals in the area via contact with materials or surfaces contaminated with the GMO and subsequent hand to mouth transmission.

150. The GMO would be imported, stored, and transported according to the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs* (TSDs) (Chapter 1, Section 2.3.2). Additionally, the GMO is supplied in an encapsulated form (beads), so unless animals were able to access and consume the beads following a spill, or the beads were damaged allowing direct access to the GMO, contact with the GMO is unlikely. In addition, biological samples that may contain GMO would also be treated in accordance with the TSDs. These practices would lower the likelihood of unintended dispersal of the GMOs.

151. As mentioned in Chapter 1, Section 2.3.8, decontamination and disinfection measures appropriate for the GMO would be carried out after administration of the GMO or in the case of accidental spills during the supply of the GMO.

152. The import, transport and storage procedures discussed above would mitigate exposure occurring because of spills of the GMO during these dealings.

Exposure during disposal of the GMO

153. Individuals may be inadvertently exposed to GMOs while disposing of used, expired, or unused vials of the GMO. The two locations where this is most likely to occur are at:

- locations where stocks of the GMO are stored,
- locations where the GMO is administered.

154. As discussed in Chapter 1, Section 2.3.8, unused and expired blister packs, vials or bottles of the GMO, as well as waste contaminated with the GMO would be treated as clinical/medical waste and disposed of in accordance with the waste disposal methods approved by the Environmental Protection Agency or Health Department in Queensland (The Queensland Government, 2025). Adherence to these procedures would reduce the likelihood of accidental exposure of people or animals to the GMO.

155. Taken together, the disposal and decontamination procedures discussed above would minimise likelihood of exposure that could be associated with conducting these dealings with the GMOs.

Potential harm

156. If people or animals are exposed to the GMOs, the GMO could establish in the gut and result in an altered microbiome. An altered microbiome could lead to persistence and long-term exposure of the GMO, which could potentially result in ill health (e.g. diarrhoea, vomiting or gut issues). As noted in Chapter 1, Section 3.4, the parent EcN could potentially have genotoxic effects due to the expression of colibactin and if the GMO established in the gut, this expression could persist. However, it is not expected that the genetic modifications would result in increased likelihood of genotoxic effects compared to the parental EcN strain. Additionally, exposure is unlikely to have negative effects of ill-health because:

- the parent organism used to generate the GMO has a long history of safe use and the modification carried out is unlikely to increase to capacity of the GMO to cause harm;
- pre-clinical studies with the GMO indicated that it did not cause severe disease and it was shown to alleviate the symptoms of IBD;

- the GMO is likely to be less effective in colonising a healthy gut than the EcN parental strain based on mouse studies;
- the GMO is less stable in the broader environment compared to the parent organism;
- although there is some literature regarding the possibility of genotoxicity associated with colibactin, which is produced the EcN parental strain, there is currently no publicly available information showing that EcN causes cancer in humans despite the probiotic has been in use for over 100 years;
- most people recover from *E. coli* infections on their own with rest and uptake of fluids to prevent dehydration;
- antibiotic treatments are available if needed.

157. As mentioned in Chapter 1, Section 3.4, the parent organism used to generate the GMO has been used in various animals without reports of any adverse events. In addition, pre-clinical studies with the GMO also did not report any adverse effects (Chapter 1, Section 4.3.3). The modifications carried out in the GMO is unlikely to increase the capacity of the GMO to cause harm in comparison to the parent organism. Therefore, the potential harm to animals is highly unlikely.

158. The use of probiotics (*Lacticaseibacillus spp, Bifidobacteria or Bacillus subtilis*) have been linked to infections, including sepsis, in immunocompromised individuals or those with pre-exsiting health conditions (Katkowska et al., 2021; Redman et al., 2014). As mentioned in Chapter 1, Section 4.3.4, there is only one report of sepsis that is attributed to EcN, this was in an infant with very low birthweight. However, it is important to note that these infections have been attributed to the consumption of probiotics by these individuals and not via transmission of the probiotics from another person. The potential exposure via transmission is likely to be at a much lower dose that the recommended dosage. Although there is limited information available regarding the possibility of *E. coli* strains to result in sepsis in immunocompromised individuals, it seems unlikely that they would be exposed to sufficient GMO to cause serious harm. However, this is an area of some uncertainty.

Conclusion

159. The potential for an unintentional exposure of people and animals to the GMO to cause harm via the alteration of the gut microbiome or genotoxicity in humans and animals is not identified as a risk that could be greater than negligible. Therefore, this risk scenario does not warrant further detailed assessment.

2.4.2 Risk Scenario 2

Risk source	GMO
Causal pathway	Administration of GMO to participant ⬇ Colonisation of the GMO in the participant's gut ⬇ Transfer of genetic material to or from the GMO ⬇ Novel GM bacteria are shed by the participant (e.g. vomit, faeces) ⬇ Exposure of medical staff, carers or pets to novel GM bacteria shed by the participant. ⬇ Colonisation of the novel GM bacteria in the gut ⬇ Infection with novel GM bacteria
Potential harm	Ill health (e.g. diarrhoea, vomiting or gut issues) or genotoxicity

Risk source

160. The source of potential harm for this postulated risk scenario is the GMO.

Causal Pathway

161. It is expected that the GMO will colonise the trial participant's gut. This could potentially result in the establishment of the GMO in the gut, reversion of the GMO to the WT phenotype through transfer of genetic material between the GMO and other bacteria colonising the participant's gut. Any *E. coli* that has reverted to wild type or strains occurring due to HGT from the GMO can then be shed by the participants and transmission of the novel GM bacteria can occur via the pathways mentioned in Risk Scenario 1.

162. As mentioned in Chapter 1, Sections 4.1 and 4.2, 2 genes have been deleted from the GMO. The deletion of Gene A reduces its ability to survive outside the gut and the deletion of Gene B by the insertion of the *ttr* gene could potentially reduce its ability to colonise a healthy gut based on mouse studies. A large scale study using human gut genomic data from over 12,000 people across 45 countries, showed that the prevalence of Enterobacteriaceae was 66% and that *Escherichia, Klebsiella* and *Enterobacter* were the most prevalent genera, as described in Chapter 1, Section 5.3. Therefore, there is a potential for the GMO to lose the *ttr* operon, which consists of structural and regulatory genes that form the tetrathionate reductase enzyme complex (Chapter 1, Section 4.1), or to reacquire each of the deleted genes via the process of horizontal gene transfer as described in Chapter 1, Section 3.5.1. However, multiple recombination events would be required for the GMO to reacquire both genes and lose the *ttr* operon (or combinations of these).

163. If the GMO reacquires both deleted genes and loses the *ttr* operon, it would have similar characteristics to the parent EcN strain that has been extensively used as a probiotic. If other

bacteria acquire the *ttr* operon, they could potentially have a survival advantage in an inflammatory environment.

164. As discussed in Chapter 1, Section 3.5.1, the main mechanisms of HGT in bacteria are transformation, transduction, transposition and conjugation. Conjugation is unlikely because the modifications are on the chromosomes of the GMO and not on plasmids.

165. Transformation involves the uptake of released DNA fragments from the environment by bacteria that are competent. In order to be competent, bacteria must have specific genes to allow them to take in DNA and receive the correct environmental signals (Blokesch, 2016). In this case, DNA fragments containing *ttr* operon could come from dead GMO. Free DNA is unlikely to survive for long in the participant's gut due to the presence of deoxyribonucleases (DNases), which are enzymes that degrade DNA. People with severe UC still show DNase activity in serum (63 ± 19%) although it is significantly less ($p < 0.001$) than that of healthy people (92 ± 11%) (Malíčková et al., 2011).

166. As described in Chapter 1, Section 4.1, the *ttr* operon from *S. enterica* is inserted into the GMO, in the genetic region of Gene B For transduction to occur, a bacteriophage must infect the GMO and then carry the *ttr* operon to a second bacteria. The bacteriophage would then need to integrate into the second bacteria's genome as a prophage. If transduction occurs, the GMO could regain Gene B, and the other bacteria will gain the *ttr* operon but lose Gene B, potentially resulting in the GMO having the ability to colonise a healthy gut similar to that of the WT, via homologous recombination. Alternatively, the *ttr* operon could be inserted in other regions in the *E. coli* and bacteriophage genome that have specific attachment sites. In the same way, the GMO can reacquire Gene A from a second bacteria.

167. Transduction does not occur at high frequency and bacteria often have defence mechanisms against bacteriophages because integration into the chromosome has the potential to kill the bacteria if it occurs in the wrong location. It is generally accepted that HGT involving chromosomes is less efficient and less common than HGT involving plasmids (circular DNA that is separate from the chromosome) (Moura de Sousa et al., 2023; Wang et al., 2023). The removal of the bacteriophage attachment sites used to insert the *ttr* operon would increase the stability of the insert by reducing the potential for transduction to occur.

168. Transposition of the *ttr* operon could possibly occur via the transfer of the *ttr* operon from the GMO to another bacteria via transposable elements or transposons, as discussed in Chapter 1, Section 3.5.1. In the same way, Gene A and B could be transferred from other bacteria to the GMO. In general, transposition is an infrequent event, probably due to its capacity for deleterious effects in the host. Usually, a transposon is translocated onto a plasmid upon conjugation. This may be followed by the integration of the transposon into the chromosome. For many transposons, however, plasmids rather than the bacterial chromosome appear to be the preferred target (Craig, 2014). The estimated rate of transposition for *E. coli* is reported to be between 3.5×10^{-4} and 1.15×10^{-5} per genome per generation (Lee et al., 2016; Sousa et al., 2013).

169. The *ttr* operon has also been isolated in a novel strain of *E. coli* and is present in 1% of the *E. coli* genomes in the NCBI database (Chapter 1, Section 5.2). Therefore, it is already present in some *E. coli* found in the microbiota of humans.

170. The applicant has also indicated that participants will be excluded from the trial if they: :

- have had a diagnosis of any non-inflammatory bowel disease related diarrhoeal illness such as bacterial or parasitic infections within the last 3 months;
- have received faecal microbiota transplantation (FMT) or other faecal-derived preparations within 6 months prior to randomisation;
- have used probiotics within 2 weeks prior to randomisation.

Therefore, the likelihood of the GMO to pass on the *ttr* operon to a pathogenic bacterium in the participant's gut is further minimised.

171. Any bacteria that receive the *ttr* operon as a result of HGT from the GMO can then be shed by the participants and transmission of the novel GM bacteria can occur via the pathways mentioned in Risk Scenario 1.

Potential harm

172. If the GMO reverts to its WT parent organism via pathways described above, it is highly unlikely to cause any harm, even in immunocompromised individuals, as discussed in Risk Scenario 1.

173. If other pathogenic bacteria in the gut were to acquire the *ttr* operon, it may give them an advantage to persist in an inflammatory environment but a potentially reduced capability of colonising a healthy human gut compared to WT. Therefore, the exposure to people with a healthy gut is unlikely to cause an increased harm. However, exposure to people experiencing inflammatory bowel disease, or have gut inflammation from any other cause, they may experience symptoms of bacterial infection (e.g. diarrhoea, stomach cramps, vomiting). However, most bacterial infections can be treated with antibiotics if needed.

Conclusion

174. The potential for unintentional exposure of people and animals to novel pathogenic GM bacteria to cause harm by ill health in humans and animals is not identified as a risk that could be greater than negligible. Therefore, this risk scenario does not warrant further detailed assessment.

2.4.3 Risk scenario 3

Risk source	GMO
Causal pathway	Administration of GMO to participant ⬇ Colonisation of the GMO in the participant's gut ⬇ (a) no modification of the GMO (b) transfer of genetic material to or from the GMO ⬇ GMO and/or novel GM bacteria are shed by the participant ⬇ GMO and/or novel GM bacteria enter wastewater system ⬇ GMO and/or novel GM bacteria survive treatment at WWTP ⬇ GMO and/or novel GM bacteria establish in the environment ⬇ A vulnerable person or animal comes into contact with the GMO and/or novel GM bacteria ⬇ GMO and/or novel GM bacteria colonises gut ⬇ Infection with GMO or novel GM bacteria.
Potential harm	Ill health (e.g. diarrhoea, vomiting or gut issues) or genotoxicity

Risk Source

175. The source of potential harm for this postulated risk scenario is the GMO.

Causal Pathway

176. The GMO or novel GM bacteria could be shed from trial participants as described in Risk Scenarios 1 and 2, including shedding in faeces, which could result in the GMO being released into the environment through wastewater. In addition, the GMO could also be released into the environment through accidental spills. This could lead to the establishment of the GMO or novel GM bacteria in the environment, which can result in exposure of people and animals (including marine or aquatic animals) to the GMO or novel GM bacteria.

177. The applicant has proposed that there would be 36 participants in the study, with 9 of those participants receiving a placebo. This limits the amount of GMO or novel GM bacteria that could be shed into the environment.

178. The GMO could also be spread into the environment through an accidental spill. Without correct decontamination with suitable disinfectants, the GMO could potentially persist on surfaces for weeks to a year (see Chapter 1, Section 3.7).

179. Accidental spills and human faecal waste, if not decontaminated appropriately, could result in the presence of the GMO or novel GM bacteria in sewage and subsequent dispersal in the aquatic environment.

180. Section 3.6 in Chapter 1 discusses the effects of wastewater treatment on bacterial populations and the use of sewerage as biosolids in agriculture in detail. This is summarised below as it is relevant to this risk scenario. In urban areas, most wastewater is processed at centralised wastewater treatment plants (WWTPs). Processes at WWTPs vary, with either 2 or 3 stages of treatments after which most wastewater is returned to the ocean, a lake, or a river.

181. Efficacy of wastewater treatment for removal of bacteria varies considerably. One UK study showed that the majority of faecal bacteria were removed at the secondary or tertiary stages of decontamination (Kay et al., 2008), with an overall reduction in bacteria of up to 3000-fold.

182. An analysis of 4 wastewater treatments plants across Australia found an average of 126 different genera of bacteria were present (Ahmed et al., 2017). The 10 most abundant genera were *Pseudomonas, Arcobacter, Bacteroides, Paludibacterium, Conchiformibius, Flavobacterium, Polynucleobacter, Acinetobacter, Parabacteroides,* and *Cloacibacterium.* A study into 4 WWTPs in Queensland found sometimes human pathogenic *E. coli* could survive tertiary treatment and reach the environment (Anastasi et al., 2010). Determining the number of *E. coli* in the environment that came from waste water is complicated by birds and other animals carrying similar *E.coli* to humans (Anastasi et al., 2012).

183. Other systems are used for waste disposal such as septic systems, aerated wastewater treatment system and dry composting toilets and participants could use non-standard toilets during activities such as camping. Such systems are generally less effective at killing bacteria than wastewater treatment plants.

184. The reduction in bacterial load and dilution of waste in larger volumes of wastewater that would occur in the wastewater treatment process mean that bacterial concentrations in areas where wastewater is released are likely to be very low. Competition and dilution still occur in other waste disposal systems but to a lower extent.

185. Additionally, the ideal temperature for most *enterobacteriaceae* is 37°C, so they are not very well adapted to cold temperatures, and do not proliferate well in waterways or the ocean (Bogosian et al., 1998). UV irradiation from the sun also kills *enterobacteriaceae.* Therefore, it is likely that the amount of GM *enterobacteriaceae* in these environments would remain low.

186. If a person, a land-based animal or a bird were to ingest water directly from an environment where the effluent is released, the amount of GMO is expected to be very low and is unlikely to be at a sufficient concentration to colonise the gut and/or to cause illness. Fish and other aquatic animals generally cannot be colonised by human gut bacteria due to their lower body temperatures (Del Rio-Rodriguez et al., 1997).

187. The modifications carried out on the GMO would not increase the ability of bacteria to survive the wastewater treatment process or its ability to survive in the environment. The GMO is likely to be outcompeted by wild-type bacteria that are not paying the metabolic cost to maintain a gene (*ttr* operon) that does not confer any advantage in the aquatic environment. In addition, a gene has been deleted from the GMO that reduces its survivability in the environment as is unable to produce an essential factor and would need rely on an external source.

188. Section 3.6 in Chapter 1, there is a potential that biosolids from sewerage plants be used for agricultural purposes. In Queensland, they would need to meet the requirements for <100 most probable number (MPN) of *E. coli* per gram of dry weight. Therefore, it is likely that the GMO could be present in the environment as biosolids. However, as the numbers of participants is low (36 including placebo controls) and the presence of other bacteria, environmental factors and modifications to the GMO that reduces its survivability in the environment, it is unlikely that the GMO or novel GM bacteria can outcompete other bacteria present in the environment and persist.

Potential harm

189. Potential harms in this risk scenario would be the same as considered in Risk scenarios 1 and 2.

Conclusion

190. The potential for the GMO to be released into the environment and result in disease in people or animals is not identified as a risk that could be greater than negligible for the same reasons as those described in Risk Scenario 1 and 2. Therefore, this risk scenario does not warrant further detailed assessment.

Section 3 Uncertainty

191. Uncertainty is an intrinsic property of risk analysis and is present in all aspects of risk analysis. This is discussed in detail in the Regulator's Risk Analysis Framework document.

192. Uncertainty is addressed by approaches such as balance of evidence, conservative assumptions, and applying risk management measures that reduce the potential for risk scenarios involving uncertainty to lead to harm. If there is residual uncertainty that is important for estimating the level of risk, the Regulator will take this uncertainty into account in making decisions.

193. As this is a first in human clinical trial, there is no available clinical biodistribution and shedding data for this GMO. Pre-clinical data using the GMO and clinical data from similar GMOs have been considered in this assessment.

194. Although the GMO is likely to produce a colibactin (a genotoxin), there is uncertainties on whether the GMO can cause cancer. However, the parent organism used to generate the GMO has been safely used as a probiotic for over 100 years. Additionally, there is no information to suggest that the genetic modification proposed for this clinical trial would impact the production of colibactin compared to the parental EcN strain. However, this remains an area of some uncertainty.

195. There is information from mouse studies indicating that the deletion of Gene B reduces the ability of the GM *E. coli* to colonise a healthy gut compared to WT, based on mouse studies. However, there is uncertainty about whether the deletion of Gene B will result in a reduced ability for the GMO to colonise a healthy human gut.

196. Overall, the level of uncertainty in this risk assessment is considered low and does not impact on the overall estimate of risk.

Section 4 Risk evaluation

197. Risk is evaluated against the objective of protecting the health and safety of people and the environment to determine the level of concern and, subsequently, the need for controls to mitigate or reduce risk. Risk evaluation may also aid consideration of whether the proposed dealings should be authorised, need further assessment, or require collection of additional information.

198. Factors used to determine which risks need treatment may include:

- risk criteria,
- level of risk,
- uncertainty associated with risk characterisation, and
- interactions between substantive risks.

199. Three risk scenarios were identified whereby the proposed dealings might give rise to harm to people or the environment. This included consideration of whether people and animals can be exposed to the GMO while conducting the dealings and whether there is a potential for HGT of the GMO with other bacteria. The potential for the GMO to be released into the environment and its effects were also considered.

200. A risk is substantive only when the risk scenario may, because of gene technology, have some chance of causing harm. Risk scenarios that do not lead to harm, or could not reasonably occur, do not represent an identified risk and do not advance in the risk assessment process.

201. In the context of the range of measures already in place, including the operating guidelines and requirements of the other regulatory agencies, and considering both the short and long term, none of these scenarios was identified as representing a substantive risk requiring further assessment. The principal reasons for this include:

- the GMO is unlikely to be shed from recipients except in faeces and vomit;
- the likelihood of accidental exposure to the GMO in people not being treated or animals would be minimised due to well-established import, transport, storage and disposal procedures;
- limited ability and opportunity for the genetic modification to be transferred by horizontal gene transfer mechanisms; and
- survival and persistence of the small amount of GMO in the Australian aquatic and terrestrial environment is highly unlikely.

202. Therefore, any risks to the health and safety of people, or the environment, from the proposed clinical trial using the GMO are considered to be negligible. The *Risk Analysis Framework* (OGTR 2013), which guides the risk assessment and risk management process, defines negligible risks as insubstantial with no present need to invoke actions for their mitigation. No controls are required to treat these negligible risks. Hence, the Regulator considers that the dealings involved in this proposed release do not pose a significant risk to either people or the environment.[2]

[2] As none of the proposed dealings are considered to pose a significant risk to people or the environment, Section 52(2)(d)(ii) of the Act mandates a minimum period of 30 days for consultation on the RARMP.

Chapter 3 Risk management plan

Section 1 Background

203. Risk management is used to protect the health and safety of people and to protect the environment by controlling or mitigating risk. The risk management plan addresses risks evaluated as requiring treatment and considers limits and controls proposed by the applicant, as well as general risk management measures. The risk management plan informs the Regulator's decision-making process and is given effect through proposed licence conditions.

204. Under section 56 of the Act, the Regulator must not issue a licence unless satisfied that any risks posed by the dealings proposed to be authorised by the licence are able to be managed in a way that protects the health and safety of people and the environment.

205. All licences are subject to 3 conditions prescribed in the Act. Section 63 of the Act requires that each licence holder inform relevant people of their obligations under the licence. The other statutory conditions allow the Regulator to maintain oversight of licensed dealings: Section 64 requires the licence holder to provide access to premises to OGTR inspectors and Section 65 requires the licence holder to report any information about risks or unintended effects of the dealing to the Regulator on becoming aware of them. Matters related to the ongoing suitability of the licence holder are also required to be reported to the Regulator.

206. The licence is also subject to any conditions imposed by the Regulator. Examples of the matters to which conditions may relate are listed in Section 62 of the Act. Licence conditions can be imposed to limit and control the scope of the dealings. In addition, the Regulator has extensive powers to monitor compliance with licence conditions under Section 152 of the Act.

Section 2 Risk treatment measures for substantive risks

207. The risk assessment of risk scenarios listed in Chapter 2 concluded that there are negligible risks to people and the environment from the proposed clinical trial with the GMO. These risk scenarios were considered in the context of the scale of the proposed clinical trial (Chapter 1, Section 2.1), the proposed controls (Chapter 1, Section 2.2), the proposed receiving environment (Chapter 1, Section 5), and considering both the short and long term effects of the GMO. Limits and controls proposed by the applicant and other general risk management measures are discussed below.

Section 3 General risk management

208. The limits and controls proposed in the application were important in establishing the context for the risk assessment and in reaching the conclusion that the risks posed to people and the environment are negligible. Therefore, to maintain the risk context, draft licence conditions have been proposed to limit the number of trial participants, limit the location of the trial to hospitals and clinical trial sites, limit the duration of the trial, as well as a range of controls to restrict the spread and persistence of the GMOs and their genetic material in the environment. The conditions are discussed and summarised in this Chapter and listed in detail in the draft licence.

3.1 Limits and controls on the clinical trial

209. Sections 2.1 and 2.2 in Chapter 1 list the limits and controls proposed by Melius. Many of these are discussed in the 3 risk scenarios considered in Chapter 2. The appropriateness of the limits and controls is considered further in the following sections.

3.1.1 Consideration of limits and controls proposed by Melius

210. The proposed clinical trial would involve a maximum of 36 participants within Australia, and dealings related to storage, preparation and administration of the GMOs would take place in medical facilities such as a hospital or clinical trial facilities in Brisbane. Activities that would occur outside of medical facilities include transport, storage and disposal of the GMOs. The applicant has proposed to complete dealings with the GMO within 5 years of commencement. A proposed licence condition limits the period when the GMO may be administered under the licence to 5 years from the date of issue of the licence. Other conditions maintaining the risk context and proposed limits of the trial such as a maximum of 36 trial participants and requirements for dealings related to preparation and administration of the GMO to be conducted at a clinical trial site are included in the draft licence. Draft licence conditions do not limit the trial to be carried out in Brisbane as any hospitals or clinical trial facilities in Australia would be appropriate to carry out this clinical trial.

211. The applicant advised that import and transport of the GMO and waste containing the GMO would be in accordance with IATA UN 3245 and the Regulator's *Guidelines for the Transport, Storage and Disposal of GMOs*, respectively. IATA UN 3373 would also meet the requirements for the import of the GMO. These are standard protocols for the handling and minimising exposure to the GMOs. Once at the clinical trial site, access to the GMO would be restricted to appropriately trained personnel. These proposed transport conditions are suitable for the GMO. Therefore, the draft licence details the minimum requirements for packaging and labelling the GMO and waste contaminated with the GMO, for transport and storage within a clinical trial site, as well as transport of the samples that may contain GMO for analysis or waste disposal. Additionally, draft conditions would require the import of the GMO should be carried out in accordance with IATA UN 3245 or UN 3373. These measures would limit the exposure of people and the environment to the GMOs.

212. Proposed inclusion and exclusion criteria for trial participants are listed in Chapter 1, Section 2.3.5. The inclusion and exclusion criteria for trial participants would be subject to approval by a HREC, who would consider the safety of the individuals involved in the trial.

213. The relevant inclusion criteria proposed by the applicant that would limit transmission include that the trial participants must:

- agree to use effective double barrier contraceptives and abstain from unprotected anal sex for the duration of the trial;
- be non-childbearing potential (women) or agree to use barrier contraceptive.

214. The relevant exclusion criteria proposed by the applicant that would limit shedding and/or HGT include:

- having a diagnosis of any non-Inflammatory bowel disease related diarrhoeal illness (e.g. *Clostridioides difficile*, coeliac disease or parasitic infections) within three months prior to randomisation;
- use of probiotics within 2 weeks prior to randomisation;
- use of agents (e.g. laxatives, anti-diarrhoeal medications and diabetic or weight loss medications) that may alter gut transit time that could lead to more shedding;
- receiving faecal microbiota transplantation (FMT) or other faecal-derived preparation within 6 months prior to randomisation;
- use of antibiotics.

215. As stated in the risk scenarios, the GMO can potentially be shed in faeces and vomit. The applicant has proposed to give participants instructions for taking samples at home where applicable. The applicant would provide sufficient containers and sealable plastic bags to ensure transport between the participant's home, and the site of analysis meet the Regulator's *Guidelines for the*

Transport, Storage and Disposal of GMOs. A condition requiring the licence holder to obtain written agreement that the trial participants would comply with the written instructions regarding sample and storage procedures is also included in the draft licence. The draft licence also requires the licence holder to provide the written instructions to the Regulator, if requested. As the predominant route of exposure is via the faecal-oral route, good hand hygiene is a key consideration in the context of the trial. Therefore, the criteria included in the draft licence are that the licence holder must provide the participants instructions of proper hand hygiene and sample collection procedures and must obtain written agreement from the participants that they are able to comply with the behavioural requirements. Additionally, the use of effective barrier contraceptives (e.g. condoms) and abstinence from unprotected anal sex would limit the potential transmission of the GMO via direct contact. Therefore, a condition is included in the draft licence to ensure the participants agree to use condoms during sexual activity.

216. Participants with non-inflammatory bowel disease related diarrhoeal illness (e.g. *Clostridioides difficile*, coeliac disease or parasitic infections); the use of agents (e.g. laxatives, anti-diarrhoeal medications and diabetic or weight loss medications) that may alter gut transit time; and antibiotics that may cause antibiotics-associated diarrhea could affect the potential shedding of the GMO. Therefore, a condition to exclude participants that have a diagnosis of non-inflammatory bowel disease within 3 months; participants that are using agents that may alter gut transit time; and participants that are using antibiotics have been included in the draft licence.

217. The presence of other bacteria in the gut may affect HGT as discussed in Risk Scenario 2. Other bacteria could be from pre-existing bacteria in the gut or from introduced bacteria (e.g. use of probiotics). Participants that potentially have diarrhoeal illness due to bacterial infections have already been excluded as mentioned in paragraph 214. This exclusion would reduce the likelihood that the GMO would encounter pathogenic bacteria for HGT to occur and result in a novel pathogenic bacterium. In general, the persistence of probiotics in the gut is low (around 2 weeks) and hence continuous dosage is required to maintain their population in the gut. Therefore, to limit the presence of other introduced bacteria, a licence condition has been included in the draft licence to exclude people that have used probiotics within the last 2 weeks.

218. FMT or other faecal-derived preparations have been used as an experimental treatment against IBD. Changes in the host-microbiome profile has been determined to last at least 300 days in healthy volunteers that have received FMT (Goloshchapov et al., 2019). Donors of FMT are usually healthy volunteers and the donor material would be screened for a wide range of bacterial and parasitic infections. Hence, it is unlikely that FMT donor material would contain pathogenic bacterium. The applicant has proposed a 6-month exclusion period for people who have received FMT. Therefore, the likelihood of the GMO producing a novel pathogenic bacterium resulting from FMT is unlikely. However, as a conservative measure, a licence condition has been included in the draft licence to exclude participants that have received FMT or other faecal derived preparation to further limit the potential introduction of other pathogenic bacteria in the gut.

219. The GMO may be shed in faeces or vomit. Although there is the potential that *E. coli* may survive wastewater treatment as discussed in Chapter 2, Section 2.4.3, the shedding of the GMO is expected to be limited (small number of patients); diluted in a large volume of wastewater; have a reduced viability in the broader environment, and limit potential to cause harm, a requirement to decontaminate the toilets after use is not included in the draft the licence. A licence condition has been included in the draft licence requiring the licence holder to provide written instructions to trial participants regarding decontamination of diarrhoea or vomit, and to obtain written agreement that trial participants will comply with these instructions to limit the dispersal of the GMO.

220. As discussed in Chapter 1, Section 5.2, infants often acquire part of their microbiome from their mothers, including through breast milk and babies born vaginally have a gut microbiome similar to the mother's birth canal. Therefore, there is some possibility of exposure of infants to the GMO

during breastfeeding and when giving birth. Additionally, the gut of an infant is often more easily colonised than adults and antibiotic use is higher in young children (Yang et al., 2016). Therefore, this risk would be minimised by excluding breastfeeding and pregnant women and a conservative approach has been taken given some areas of uncertainty about the GMO, and as such a condition to exclude pregnant and breastfeeding women from the clinical trial has been included in the draft licence.

221. The clinical staff preparing and observing the administration of the GMO of participants would wear PPE including gown and gloves. Additional PPE (masks and eye protection) would be worn when cleaning any accidental spills or potential shedding of the GMO via contaminated faeces, vomit or rupture of samples with decontaminants that are effective against the GMO. These practices would minimise exposure of people handling and administering the GMOs (Risk scenario 1) and have been included in the draft licence conditions.

222. Conditions are included in the draft licence requiring the licence holder to ensure that all GMOs within the clinical trial site, including material or waste that has been in contact with the GMO, are decontaminated by autoclaving, chemical treatment or by high-temperature incineration. Draft licence conditions require the licence holder to ensure that the GMO, or material or waste that has been in contact with the GMO, to be destroyed by external service providers is done through a clinical waste stream. This is considered satisfactory, provided that the licence holder is only permitted to engage persons who can adhere to appropriate standards to conduct the dealings.

223. The Industry Code of Practice for the Management of Clinical and Related Wastes details requirements for clinical waste including waste segregation, packaging, labelling, storage, transport and accountability (Biohazard Waste Industry, 2010). The clinical waste stream typically involves destruction of infectious waste by incineration or autoclaving, which are considered appropriate for disposal of the GMO. Given that *E. coli* can persist in the environment, disposal measures such as burial or maceration would not ensure containment. Therefore, the draft licence requires waste disposal by external service providers to be by autoclaving or high-temperature incineration. These measures would limit the exposure of people or other animals to the GMOs.

224. A standard condition is included in the draft licence requiring that the licence holder to ensures dealings are conducted so as to ensure containment of the GMO, not compromise the health and safety of people and minimise unintentional exposure to the GMO. A note to the condition explains that compliance may be achieved by only engaging persons who are required to adhere to appropriate standards to conduct the dealings.

225. Other standard conditions included in the draft licence state that only people authorised by the licence holder are covered by the licence, and that the licence holder must inform all people dealing with the GMOs, other than external service providers, of applicable licence conditions.

226. Further conditions to be implemented in the draft licence is to ensure that a compliance management plan is in place for each clinical trial site before administration of the GMOs commences at that site. The compliance management plan must detail how the licence holder intends to comply with the licence conditions, including listing persons responsible for site management, proposed reporting structures, staff training procedures and transport and disposal processes.

3.1.2 Summary of licence conditions to be implemented to limit and control the clinical trial

227. Licence conditions have been drafted to limit and control the proposed clinical trial, based on the above considerations. These include requirements to:

- limit the trial to 36 trial participants;
- conduct the trial at suitable clinical trial sites;
- limit the time when the GMO can be administered to 5 years from issue of the licence;

- restrict access to the GMO;
- ensure personnel involved in the trial are appropriately trained and follow appropriate behavioural requirements;
- ensure appropriate PPE is used;
- restrict personnel permitted to administer the GMO;
- appropriately decontaminated the GMO and materials and equipment that have been in contact with the GMO;
- transport and store the GMO and samples from GMO-treated participants in accordance with IATA shipping classification UN 3245 or UN 3373 [Category B] and/or the minimum requirements for packaging, and labelling as detailed in the draft licence;
- use the clinical waste stream when external service providers are used to destroy unused GMO and GMO-related waste.

3.2 Other risk management considerations

228. All DIR licences issued by the Regulator contain several conditions that relate to general risk management. These include conditions relating to:

- applicant suitability
- contingency plans
- identification of the persons or classes of persons covered by the licence
- reporting requirements
- access for the purpose of monitoring for compliance.

3.2.1 Applicant suitability

229. In making a decision whether or not to issue a licence, the Regulator must have regard to the suitability of the applicant to hold a licence. Under Section 58 of the Act, matters that the Regulator must take into account include:

- any relevant convictions of the applicant
- any revocation or suspension of a relevant licence or permit held by the applicant under a law of the Commonwealth, a State or a foreign country
- the capacity of the applicant to meet the conditions of the licence.

230. If a licence were issued, the conditions would include a requirement for the licence holder to inform the Regulator of any information that would affect their suitability.

231. In addition, the applicant organisation must have access to an IBC and be an accredited organisation under the Act.

3.2.2 Contingency plans

232. Should a licence be issued, Melius would be required to submit a contingency plan to the Regulator before commencing dealings with the GMOs. This plan would detail measures to be undertaken in the event of:

- the unintended release of the GMOs, including spills
- exposure of, or transmission to persons other than trial participants
- a person exposed to the GMOs developing a serious adverse response.

3.2.3 Identification of the persons or classes of persons covered by the licence

233. If issued, the persons covered by the licence would be the licence holder and employees, agents or contractors of the licence holder and other persons who are, or have been, engaged or otherwise authorised by the licence holder to undertake any activity in connection with the dealings authorised by the licence. Prior to dealings with the GMOs, Melius is required to provide a list of

people and organisations that are covered by the licence, or the function or position where names are not known at the time.

3.2.4 Reporting requirements

234. If issued, the licence would require the licence holder to immediately report any of the following to the Regulator:

- any additional information regarding risks to the health and safety of people or the environment associated with the dealings
- any contraventions of the licence by persons covered by the licence
- any unintended effects of the clinical trial.

235. Several written notices would also be required under the licence regarding dealings with the GMO, to assist the Regulator in designing and implementing a monitoring program for all licensed dealings. The notices include:

- identification of the clinical trial sites where administration of the GMO to trial participants would take place
- expected date of administration with the GMOs for each clinical trial site
- cease of administration with the GMOs for each clinical trial site.

3.2.5 Monitoring for compliance

236. The Act stipulates, as a condition of every licence, that a person who is authorised by the licence to deal with a GMO, and who is required to comply with a condition of the licence, must allow inspectors and other persons authorised by the Regulator to enter premises where a dealing is being undertaken for the purpose of monitoring or auditing the dealing.

237. If monitoring activities identify changes in the risks associated with the authorised dealings, the Regulator may also vary licence conditions, or if necessary, suspend or cancel the licence.

238. In cases of non-compliance with licence conditions, the Regulator may instigate an investigation to determine the nature and extent of non-compliance. The Act provides for criminal sanctions of large fines and/or imprisonment for failing to abide by the legislation, conditions of the licence or directions from the Regulator, especially where significant damage to the health and safety of people or the environment could result.

Section 4 Issues to be addressed for future releases

239. Additional information has been identified that may be required to assess an application for a commercial release of the GMO, or to justify a reduction in limits and controls. This includes:

- information and data that would address the uncertainties noted in Chapter 2, Section 3. Specifically, information obtained on the biodistribution and shedding of the GMOs in trial participants and the potential of the GMO to cause genotoxicity.

Section 5 Conclusions of the consultation RARMP

240. The risk assessment concludes that the proposed clinical trial of the GMOs poses negligible risks to the health and safety of people or the environment as a result of gene technology. These negligible risks do not require specific risk treatment measures.

241. If a licence is issued, conditions are imposed to limit the trial to the proposed scale, location and duration, and to restrict the spread and persistence of the GMOs and its genetic material in the environment, as these were important considerations in establishing the context for assessing the risks.

Chapter 4 Draft licence conditions

Section 1 Interpretations and definitions

1. In this licence:

    (a) unless defined otherwise in this licence, words and phrases used in this licence have the same meaning as they do in the Act and the Gene Technology Regulations 2001;

    (b) words importing a gender include every other gender;

    (c) words in the singular number include the plural and words in the plural include the singular;

    (d) expressions used to denote persons generally (such as “person”, “party”, “someone”, “anyone”, “no one”, “one”, “another” and “whoever”), include a body politic or corporate as well as an individual;

    (e) references to any statute or other legislation (whether primary or subordinate) are a reference to a statute or other legislation of the Commonwealth of Australia as amended or replaced from time to time and equivalent provisions, if any, in corresponding State law, unless the contrary intention appears;

    (f) where any word or phrase is given a particular meaning, other grammatical forms of that word or phrase have corresponding meanings;

    (g) specific conditions prevail over general conditions to the extent of any inconsistency.

2. In this licence:

‘Act’ means the *Gene Technology Act 2000* (Commonwealth) or the corresponding State Law under which this licence is issued.

‘Analytical facility’ means a laboratory in Australia accredited to undertake testing of human diagnostic Samples, such as a medical testing laboratory accredited by the National Pathology Accreditation Advisory Council (NPAAC).

‘Clinical trial site’ means a medical facility in Australia such as a clinical trial facility and associated Pharmacy, which are notified in writing to the Regulator for the purposes of conducting this clinical trial.

‘Decontaminate’ (or ***‘Decontamination’***) means, as the case requires, kill the GMOs by one or more of the following methods:

(a) chemical treatment;

(b) autoclaving;

(c) high-temperature incineration; or

(d) a method approved in writing by the Regulator.

Note: 'As the case requires' has the effect that, depending on the circumstances, one or more of these techniques may not be appropriate.

‘External service provider’ means a person engaged by the licence holder solely in relation to transport, storage and/or disposal of the GMOs, or Sample analysis other than at a Clinical trial site, and who is not undertaking any dealings with the GMOs that are not for those purposes.

‘GM’ means genetically modified.

‘*GMO*’ means the genetically modified organisms that are the subject of the dealings authorised by this licence.

'NLRD' is a Notifiable low risk dealing. Dealings conducted as an NLRD must be assessed by an institutional biosafety committee (IBC) before commencement and must comply with the requirements of the Gene Technology Regulations 2001.

'OGTR' means the Office of the Gene Technology Regulator

'Personal information' has the same meaning as in the *Privacy Act 1988*. Personal information means information or an opinion about an identified individual, or an individual who is reasonably identifiable:

(a) whether the information or opinion is true or not; and

(b) whether the information or opinion is recorded in a material form or not.

'Pharmacy' means a location within the Clinical trial site, where authorised staff stores, prepares, and dispenses medications in a medical environment.

'*Regulations'* means the *Gene Technology Regulations 2001* (Commonwealth) or the corresponding State Law under which this licence is issued.

'Regulator' means the Gene Technology Regulator.

'Sample' means any biological material collected from a treated trial participant for analysis as part of the trial.

Section 2 General conditions and obligations

Holder of licence

3. The licence holder is Melius MicroBiomics Pty Ltd.

Remaining an Accredited Organisation

4. The licence holder must, at all times, remain an accredited organisation.

Validity of licence

5. This licence remains in force until it is suspended, cancelled or surrendered. No dealings with the GMO are authorised during any period of suspension, or after the licence has been cancelled or surrendered.

Note: Although this licence has no expiry date, the duration of preparation and administration of the GMOs is restricted in accordance with Condition 23.

Persons covered by this licence

6. The persons covered by this licence are:

(a) the licence holder, and any employees, agents or External service providers engaged by the licence holder; and

(b) the project supervisor(s); and

(c) other persons who are, or have been, engaged or otherwise authorised by the licence holder or the project supervisor to conduct any of the dealings authorised by this licence.

7. To the extent that any activity by a trial participant may be considered to be a dealing with the GMO as described in **Attachment A** for purposes of the Act, that dealing is authorised by this licence.

8. The licence holder must keep a record of all persons covered by this licence, and must keep a record of the contact details of the project supervisor(s) for the licence.

Note: Where External service providers are used, it is sufficient to record the company name and the position or job title of the person(s) conducting the dealing.

9. The licence holder must provide information related to the persons covered by the licence when requested to do so in writing by the Regulator and must provide the information within a time period stipulated by the Regulator.

Description of GMOs covered

10. The licence authorises specific dealings in respect of the GMO identified and described in **Attachment A**.

Dealings authorised by this licence

11. The licence holder and persons covered by this licence may conduct the following dealings with the GMO:

(a) import the GMO;

(b) conduct the following experiments with the GMOs:

i) prepare the GMO for administration to trial participants;

ii) oral administration of the GMO to trial participants;

iii) collect Samples from trial participants;

iv) analyse the Samples described in 11(b)iii);

(c) transport the GMO; and

(d) dispose of the GMOs;

and may possess, supply, use or store the GMO for the purposes of, or in the course of, any of these dealings.

12. Supply of the GMOs for the purposes of dealings to any other person or organisation not covered by this licence is only authorised by this licence if the Regulator provides prior written approval to the licence holder.

Note: For approval to be granted, the receiving person or organisation must have an appropriate authorisation to conduct dealings with the GMOs. This is likely to be an NLRD or a licence issued by the Regulator.

Conditions imposed by the Act

Note: The Act mandates the following 3 conditions.

Informing people of licence conditions (section 63)

13. The licence holder must inform any person covered by the licence, to whom a particular condition of the licence applies, of the following:

(a) the particular condition, including any variations of it; and

(b) the cancellation or suspension of the licence; and

(c) the surrender of the licence.

Note: No particular conditions of this licence apply to trial participants; therefore, Condition 13 does not apply to trial participants.

Monitoring and audits (section 64)

14. If a person is authorised by this licence to deal with the GMO and a particular condition of this licence applies to the dealing by that person, the person must allow the Regulator, or a person authorised by the Regulator, to enter premises where the dealing is being undertaken, for the purposes of auditing or monitoring the dealing.

Additional information to be given to the Regulator (section 65)

15. The licence holder must immediately inform the Regulator, if they become aware of:

(a) additional information about any risks to the health and safety of people, or to the environment, associated with the dealings authorised by the licence; or

(b) any contraventions of the licence by a person covered by the licence; or

(c) any unintended effects of the dealings authorised by the licence.

Note 1: For the purposes of this Condition:

(a) The licence holder is taken to have become aware of additional information if they were reckless as to whether such information existed; and

(b) The licence holder is taken to have become aware of contraventions, or unintended effects, if they were reckless as to whether such contraventions had occurred, or such unintended effects existed.

Note 2: Contraventions of the licence may occur through the action or inaction of a person.

Note 3: Additional information includes any changes at a Clinical trial site, which might increase the likelihood of unintentional exposure of people or release of the GMO into the environment.

Note 4: An example of informing immediately is contact made at the time of the incident via the OGTR free call phone number 1800 181 030 or email to OGTR.M&C@health.go.au.

Informing the Regulator of any material changes of circumstance

16. The licence holder must immediately, by notice in writing, inform the Regulator of:

(a) any relevant conviction of the licence holder occurring after the commencement of this licence;

(b) any revocation or suspension after the commencement of this licence, of a licence or permit held by the licence holder under a law of the Commonwealth, a State or a foreign country, being a law relating to the health and safety of people or the environment;

(c) any event or circumstances occurring after the commencement of this licence that would affect the capacity of the licence holder to meet the conditions in it.

17. The licence holder must provide information related to the licence holder's ongoing suitability to hold a licence when requested to do so in writing by the Regulator and must provide the information within a time period stipulated by the Regulator.

Further conditions with respect to informing persons covered by the licence

18. If a particular condition, including any variation of it, applies to an External service provider covered by this licence, the licence holder must not permit that person to conduct any dealings unless the person has been informed of the condition, including any variation to it.

Note: Information required under Condition 18 may be provided to External service providers who are engaged solely for storage and transport of the GMO through labelling of the outermost container of the GMOs in accordance with Condition 36(a).

19. If a particular condition, including any variation of it, applies to a person with respect to any dealing, other than to an External service provider, the licence holder must not permit a person covered by this licence to conduct that dealing unless:

(a) the licence holder has obtained from the person a signed and dated statement that the person:

i) has been informed by the licence holder of the condition and, when applicable, its variation; and

ii) has understood and agreed to be bound by the condition, or its variation; and

iii) has been trained in accordance with sub-condition 18(b) below; and

(b) the licence holder has trained that person in a manner which enables them to conduct the dealings in accordance with the conditions of this licence.

20. The licence holder must notify all persons covered by the licence, from whom Personal information relevant to the administration and/or enforcement of the licence is collected by the licence holder, that such Personal information may be disclosed to the Regulator.

21. The licence holder must ensure that a copy of the licence is readily available to all persons covered by the licence, other than External service providers, who are conducting dealings with the GMO.

Note: The licence may be made available electronically.

Section 3 Limits and control measures

Limits on clinical trials conducted under this licence

22. The GMO may be administered to a maximum of 36 trial participants.

23. The preparation and administration of the GMO must be completed within 5 years from the date of issuing of the licence.

Preparation and administration of the GMO

24. Administration of the GMO to trial participants must not commence prior to approval by a Human Research Ethics Committee.

25. The following activities must occur within a Clinical trial site:

(a) preparation of the GMO for administration to trial participants; and

(b) administration of the GMO to trial participants.

Note: Before any of these activities take place, the details of each Clinical trial site must have been notified to the Regulator in accordance with Condition 41(a).

Conditions relating to trial participants

26. The licence holder must notify each trial participant, from whom Personal information relevant to the administration and/or enforcement of the licence is collected by the licence holder, that such Personal information may be disclosed to the Regulator.

27. The licence holder must ensure that exclusion criteria used in selecting trial participants include (though are not limited to) the following persons:

(a) those who are pregnant or breastfeeding;

(b) those having a diagnosis of any non-Inflammatory bowel disease related diarrhoeal illness (e.g. *Clostridioides difficile*, coeliac disease or parasitic infections) within three months prior to administration;

(c) those who have used probiotics within 2 weeks prior to administration;

(d) those using agents that can alter gut transit time (e.g. laxatives, anti-diarrhoeal medications and diabetic or weight loss medications);

(e) those who have received faecal microbiota transplantation (FMT) or other faecal-derived preparation within 6 months prior to administration; and

(f) those who are currently using antibiotics.

28. Before inoculating any trial participant with the GMO, the licence holder must obtain written agreement from the trial participant that they will:

(a) use condoms during sexual activity for 60 days following each administration of the GMO; and

(b) agree to comply with the written instructions provided by the licence holder regarding:

i) good hand hygiene (e.g. frequent hand washing with soap or hand disinfectant);

ii) sample collection and storage procedures; and

iii) decontamination procedures in the event of diarrhoea or vomiting.

(c) upon discharge from the hospital, agree to collect and return any Samples in containers and waste in sealable plastic bags provided by the licence holder, according to the instructions provided prior to administration.

29. The wording of the written instructions provided to trial participants in accordance with Condition 28 (b) and (c) must be provided to the Regulator upon request.

Note: Condition 28(a) is intended to minimise physical contact or exchange of bodily fluids during sexual activity.

Conditions related to the conduct of the dealings

30. Conditions that apply to dealings with GMOs do not apply to:

(a) certain Samples[3] not containing the GMO; and

(b) other Samples, materials and waste, that are reasonably expected not to contain the GMO. Upon request from the Regulator, the licence holder must provide a written justification for this expectation.

31. The licence holder must ensure that dealings are only conducted in a manner which:

(a) does not compromise the health and safety of people; and

(b) minimises the exposure of persons conducting the dealings to the GMO, other than intended exposure of trial participants.

Note: The licence holder may do this by only engaging or otherwise authorising persons to conduct dealings who are required to adhere to appropriate standards and guidelines. For example, standards developed by the National Pathology Accreditation Advisory Council for pathology practices, the Australian Guidelines for the Prevention and Control of Infection in Healthcare, Guidelines for Good Clinical Practice and the National Safety and Quality Health Service (NSQHS) Standards.

32. The licence holder must ensure that procedures are in place to account for the GMO from import to destruction/export, and records must be made available the Regulator on request.

[3] Confidential Commercial Information: Some details about the samples collected have been declared as Confidential Commercial Information under section 185 of the Act. This information will be made available to the prescribed experts and agencies that will be consulted on this application. CCI is not available to the public.

Work practices at Clinical trial sites

33. For the purposes of Condition 31, the work practices and behaviours within a Clinical trial site must include, but are not limited to, the following:

(a) persons preparing the GMO must wear personal protective equipment (PPE), including gowns and gloves;

(b) persons cleaning up a spill of the GMO or potential shedding (e.g. faeces, vomit, ruptured beads) must wear personal protective equipment (PPE), including gowns, gloves, mask and eye protection; and

(c) preparation and supervision of the administration of the GMO must be conducted by suitably qualified and trained staff.

Transport, storage and disposal of the GMOs

34. Unless covered by an NLRD, the licence holder must ensure that transport of the GMOs is conducted only for the purposes of, or in the course of, another dealing permitted by this licence.

35. For the purposes of import, transport between the border and a Clinical trial site, the licence holder must ensure the GMO is packaged, labelled, stored and transported consistent with International Air Transport Association (IATA) shipping classification UN 3245 or UN 3373.

36. The licence holder must ensure that transport and storage of the GMO, unless conducted according to Condition 34 or 35 follows these sub-conditions:

(a) The GMO must be contained within sealed, unbreakable primary and secondary containers, with the outer packaging labelled to indicate at least:

i) that it contains GMOs; and

ii) that it contains biohazardous material as designated by a biohazard label; and

iii) the contact details for the licence holder; and

iv) instructions to notify the licence holder in case of loss or spill of the GMOs; and

v) the external surface of the primary and secondary containers must be decontaminated prior to and after transport; and

vi) procedures must be in place to ensure that GMO can be accounted for and that a loss of GMOs during transport or storage or failure of delivery can be detected; and

vii) access to the GMO is restricted to authorised persons for whom Condition 18 or Condition 19 has been met (i.e. the GMO is within a locked unit or an area which has restricted access). This includes situations where containers are left for collection in a holding area, or left unattended prior to Decontamination; and

Note: All stored GMOs remain the responsibility of the licence holder.

viii) if the GMO is being transported or stored with a coolant (e.g. dry ice, liquid nitrogen or any other coolant) which will release a gas, a mechanism to allow the escape of the gas must be included. If water ice is used as a coolant then the outer packaging should be constructed so as to prevent any leakage. All containers must be able to withstand the temperatures to which they will be subjected; and

Note: When transporting and storing with coolants, it is preferable for coolants to be used outside of the secondary container.

ix) a consolidated record of all GMOs being stored under this condition is maintained and made available to the Regulator upon request; and

x) for the purposes of transport entirely within a building, where the GMO is accompanied by an authorised person for whom Condition 19 has been met, Conditions 36(a)iii), 36(a)iv) and 36vi) do not apply.

37. The licence holder must ensure that all GMOs and waste reasonably expected to contain the GMOs are Decontaminated:

(a) prior to disposal, unless the method of disposal is also a method of Decontamination; and

(b) before or upon suspension, cancellation or surrender of the licence, unless covered by another authorisation under the Act, or exported; and

(c) by autoclaving, chemical treatment, high-temperature incineration or any other method approved in writing by the Regulator.

38. Where transport is conducted by External service providers for the purposes of destruction, the licence holder must ensure that the GMO, or waste reasonably expected to contain the GMO, enters the clinical waste stream for Decontamination via autoclaving or high-temperature incineration.

Note: In the event of a spill during transport by an External service provider, compliance with relevant State or Territory legislation and regulations to manage clinical or biohazardous spills is sufficient.

Contingency plans

39. The licence holder must ensure that any person (other than a trial participant) exposed to the GMOs via oral ingestion is offered prompt medical advice. The clinician must be provided with any relevant information about the GMO.

40. If there is a spill or an unintentional release of GMO at a Clinical trial site, the following measures must be implemented:

(a) the GMOs must be contained to prevent further dispersal; and

(b) persons cleaning up the GMO must wear appropriate PPE in accordance with Condition 33(b); and

(c) the exposed area must be decontaminated with an appropriate chemical disinfectant effective against the GMO; and

(d) any material used to clean up the spill or PPE worn during clean-up of the spill must be Decontaminated; and

(e) the licence holder must be notified as soon as reasonably possible.

Section 4 Reporting and Documentation

Note: The following licence conditions are imposed to demonstrate compliance with other conditions and facilitate monitoring of compliance by staff of the OGTR. Notices and reports may be by email to OGTR.M&C@health.gov.au. A summary of notification and reporting requirements is provided at ***Attachment B****.*

41. At least 14 days prior to first administering the GMO at each Clinical trial site, or a timeframe agreed to in writing by the Regulator, the licence holder must provide the Regulator with a Compliance Management Plan for that Clinical trial site, specifying:

(a) the name, address and description of the Clinical trial site, including any associated Pharmacies/storage areas/Analytical facilities/hospitals;

(b) the role and contact details for key persons responsible for the management of the trial at the site;

(c) that the Institutional Biosafety Committee (IBC) associated with the site (if any) has been notified of the trial and have been consulted regarding site specific procedures;

(d) the proposed reporting structure for the trial at the site and how the reporting structure enables the licence holder to become aware of all reportable events including but not limited to Condition 15, 16, 42 and 43;

(e) details of how the persons covered by the licence (for that type of dealing) will be informed of licence conditions applicable to them and how they will be trained to safely conduct the dealings;

(f) the person(s) or class of persons administering the GMO;

(g) where, within the site, the GMO is expected to be administered;

(h) the expected date of first administration; and

(i) how compliance with Condition 31 will be achieved in relation to preparation of participant Samples for analysis subsequent to administering the GMO.

Note: For the purpose of finding out whether the Act has been complied with, an OGTR inspector may, if entry is at a reasonable time, enter a facility occupied by the licence holder or a person covered by the licence and exercise monitoring powers.

42. For each Clinical trial site, the licence holder must notify the Regulator, in writing, of the end of the clinical trial, no later than 30 days:

(a) the final dose being administered; or

(b) the decision that no further participants will be treated at the site.

43. The licence holder must inform the Regulator as soon as reasonably possible:

(a) in the event of a loss or spill of the GMO;

(b) in the event of the exposure of a person other than a trial participant to the GMO via oral ingestion; and

(c) if a trial participant has not followed the procedures described in the instructions provided by the licence holder.

44. Upon request from the Regulator, the licence holder must provide any signed records or documentation collected under a condition of this licence, within a time period stipulated by the Regulator.

Attachment A

DIR No: 221

Full Title: Clinical trial with a genetically modified *E. coli* for the treatment of ulcerative colitis

Licence holder

Melius MicroBiomics Pty Ltd

GMO Description

GMOs covered by this licence:

Escherichia coli NIssle strain 1917 modified by the insertion of the tetrathionate reductase (*ttr*) operon from *Salmonella enterica* Typhimurium and deletion of 2 genes.

Parent Organisms:

Common Name: *Escherichia coli*

Scientific Name: *Escherichia coli* Nissle strain 1917

Modified traits:

Categories: Human therapeutic

Description: The GMO is a probiotic strain that has been modified to increase its capability of colonising the gut in inflammatory conditions typical of patients with ulcerative colitis. It also has been modified by the deletion of 2 genes that reduce its ability to survive outside the gut and potentially reducing its ability to colonise a healthy gut. Modified genes and regulatory sequences are listed in Table 1.

Table 1. Nucleic acid responsible for conferring the modified traits

Identity and modifications	• Insertion of 2 copies of the tetrathionate reductase (*ttr*) operon from *Salmonella enterica* • Deletion of 2 genes (Genes A and B) from the parent organism[4]
Function	• *ttr* operon – increased ability to colonise inflamed gut • Gene A – reduced survival in the broader environment • Gene B – potential reduced ability to colonise the healthy gut compared to WT.

Trial participants and route of administration of the GMO

Oral ingestion by healthy adult humans and patients with ulcerative colitis.

[4] Confidential Commercial Information: Some details about the modification in GM *E. coli* have been declared as Confidential Commercial Information under section 185 of the Act. This information will be made available to the prescribed experts and agencies that will be consulted on this application. CCI is not available to the public.

Attachment B

Prior to the commencement of the trial	Condition	Timeframe for reporting
A written Compliance Management Plan for each Clinical trial site: • the name, address and description of the Clinical trial site, including any associated Pharmacies/storage areas/Analytical facilities; • the role and contact details for key persons responsible for the management of the trial at the site; • that the Institutional Biosafety Committee (IBC) associated with the site (if any) has been notified of the trial and have been consulted regarding site specific procedures; • the proposed reporting structure for the trial at the site and how the reporting structure enables the licence holder to become aware of all reportable events; • details of how the persons covered by the licence (for that type of dealing) will be informed of licence conditions applicable to them and how they will be trained to safely conduct the dealings; • the person(s) or class of persons administering the GMO; • where, within the site, the GMO is expected to be administered; • expected date of first administration; and • how compliance with Condition 31 will be achieved in relation to preparation of participant Samples for analysis subsequent to administering the GMO	41	At least 14 days prior to the first administration of the GMO at each Clinical trial site, or a timeframe agreed to in writing by the Regulator
Information to be provided at any time during the Clinical trial		
Any additional information related to the health and safety of people and the environment associated with the dealing covered by the licence, or any unintended effect of the dealing authorised by the licence	15(a), (c)	Immediately
Information related to any contravention of the licence by a person covered by the licence	15(b)	Immediately
Any relevant conviction of the licence holder	16(a)	Immediately

Any revocation or suspension of a licence or permit held by the licence holder under a law of the Commonwealth, a State or a foreign country	16(b)	Immediately
Any event or circumstances that would impact the licence holder capacity to meet the licence conditions	16(c)	Immediately
Provide notification to the Regulator, in writing, of the final GMO administration of the last trial participant at each Clinical trial site	42(a), (b)	Within 30 days of the decision to cease GMO administration at that particular Clinical trial site.
Any loss or spill of the GMO, or exposure of a person other than the trial participant to the GMO	43(a), (b)	As soon as reasonably possible
Any event where a trial participant has not followed the procedures described in the instruction provided by the licence holder	43(c)	As soon as reasonably possible
Information to be provided on request by the Regulator		
Information related to the persons covered by the licence	9	Within a timeframe stipulated by the Regulator
Information related to the licence holder's ongoing suitability to hold a licence	17	Within a timeframe stipulated by the Regulator
The wording of the written instructions provided to trial participants in accordance with Condition 28(b) and (c)	28 (b), (c)	Within a timeframe stipulated by the Regulator
Copies of signed and dated statements and training records	19	Within a timeframe stipulated by the Regulator
A consolidated record of all GMOs being stored	36(ix)	Within a timeframe stipulated by the Regulator
Any signed records or documentation collected under a condition of this licence	44	Within a timeframe stipulated by the Regulator

References

AAP (2018). Report warns superbug *E. coli* driving Australia sepsis rates. (HealthTimes: https://healthtimes.com.au/hub/infection-control/33/news/aap/report-warns-superbug-ecoli-driving-australia-sepsis-rates/3202/). https://healthtimes.com.au/hub/infection-control/33/news/aap/report-warns-superbug-ecoli-driving-australia-sepsis-rates/3202/.

Abram, K., Udaondo, Z., Bleker, C., Wanchai, V., Wassenaar, T.M., Robeson, M.S., 2nd, and Ussery, D.W. (2021). Mash-based analyses of *Escherichia coli* genomes reveal 14 distinct phylogroups. Commun Biol *4*, 117.

Adsit, F.G., Jr., Randall, T.A., Locklear, J., and Kurtz, D.M. (2022). The emergence of the tetrathionate reductase operon in the *Escherichia coli/Shigella* pan-genome. Microbiologyopen *11*, e1333.

Ahmed, W., Staley, C., Sidhu, J., Sadowsky, M., and Toze, S. (2017). Amplicon-based profiling of bacteria in raw and secondary treated wastewater from treatment plants across Australia. Applied microbiology and biotechnology *101*, 1253-1266.

Anastasi, E.M., Matthews, B., Gundogdu, A., Vollmerhausen, T.L., Ramos, N.L., Stratton, H., Ahmed, W*., et al.* (2010). Prevalence and persistence of *Escherichia coli* strains with uropathogenic virulence characteristics in sewage treatment plants. Appl Environ Microbiol *76*, 5882-5886.

Anastasi, E.M., Matthews, B., Stratton, H.M., and Katouli, M. (2012). Pathogenic *Escherichia coli* found in sewage treatment plants and environmental waters. Appl Environ Microbiol *78*, 5536-5541.

Auvray, F., Perrat, A., Arimizu, Y., Chagneau, C.V., Bossuet-Greif, N., Massip, C., Brugère, H*., et al.* (2021). Insights into the acquisition of the *pks* island and production of colibactin in the *Escherichia coli* population. Microbial Genomics *7*.

Berg, C.M., Grullon, C.A., Wang, A., Whalen, W.A., and Berg, D.E. (1983). Transductional instability of Tn5-induced mutations: Generalized and specialized transduction of Tn5 by bacteriophage P1. Genetics *105*, 259-263.

Biohazard Waste Industry (2010). Industry Code of Practice for the Management of Clinical and Related Wastes, 6 edn.

Blokesch, M. (2016). Natural competence for transformation. Current Biology *26*, R1126-R1130.

Blum-Oehler, G., Oswald, S., Eiteljörge, K., Sonnenborn, U., Schulze, J., Kruis, W., and Hacker, J. (2003). Development of strain-specific PCR reactions for the detection of the probiotic *Escherichia coli* strain Nissle 1917 in fecal samples. Research in Microbiology *154*, 59-66.

Bogosian, G., Morris, P.J.L., and O'Neil, J.P. (1998). A Mixed Culture Recovery Method Indicates that Enteric Bacteria Do Not Enter the Viable but Nonculturable State. Appl Environ Microbiol *64*, 1736-1742.

Bureau of Meteorology (2024). National performance report 2023–24: urban water utilities, part B. (Melbourne: Bureau of Meteorology).

Chen, H., Lei, P., Ji, H., Yang, Q., Peng, B., Ma, J., Fang, Y*., et al.* (2023). Advances in *Escherichia coli* Nissle 1917 as a customizable drug delivery system for disease treatment and diagnosis strategies. Mater Today Bio *18*, 100543.

Chung, P.Y. (2016). The emerging problems of *Klebsiella pneumoniae* infections: carbapenem resistance and biofilm formation. FEMS Microbiology Letters *363*.

Cobo-Simón, M., Hart, R., and Ochman, H. (2023). *Escherichia Coli*: What Is and Which Are? Mol Biol Evol *40*.

Craig, N. (2014). Molecular Biology - Principles of Genome Function, 2nd Edition edn.

Cummings, J.H., Bingham, S.A., Heaton, K.W., and Eastwood, M.A. (1992). Fecal weight, colon cancer risk, and dietary intake of nonstarch polysaccharides (dietary fiber). Gastroenterology *103*, 1783-1789.

Darvodelsky, P. (2012). Biosolids snapshot. (Department of Sustainability, Environment, Water, Population and Communities).

Del Rio-Rodriguez, R.E., Inglis, V., and Millar, S.D. (1997). Survival of *Escherichia coli* in the intestine of fish. *28*, 257-264.

Department of Environment and Science (2019). End of Waste Code: Biosolids. (State of Queensland).

Dionisio, F., Zilhão, R., and Gama, J.A. (2019). Interactions between plasmids and other mobile genetic elements affect their transmission and persistence. Plasmid *102*, 29-36.

Dubbert, S., Klinkert, B., Schimiczek, M., Wassenaar, T.M., and Bunau, R.V. (2020). No Genotoxicity Is Detectable for *Escherichia coli* Strain Nissle 1917 by Standard *In Vitro* and *In Vivo* Tests. Eur J Microbiol Immunol (Bp) *10*, 11-19.

Falzone, L., Lavoro, A., Candido, S., Salmeri, M., Zanghi, A., and Libra, M. (2024). Benefits and concerns of probiotics: an overview of the potential genotoxicity of the colibactin-producing *Escherichia coli* Nissle 1917 strain. Gut Microbes *16*, 2397874.

Fang, K., Jin, X., and Hong, S.H. (2018). Probiotic *Escherichia coli* inhibits biofilm formation of pathogenic *E. coli* via extracellular activity of DegP. Scientific Reports *8*, 4939.

Fang, M., Zhang, R., Wang, C., Liu, Z., Fei, M., Tang, B., Yang, H.*, et al.* (2024). Engineering probiotic *Escherichia coli* Nissle 1917 to block transfer of multiple antibiotic resistance genes by exploiting a type I CRISPR-Cas system. Appl Environ Microbiol *90*, e0081124.

Frazão, N., Sousa, A., Lässig, M., and Gordo, I. (2019). Horizontal gene transfer overrides mutation in *Escherichia coli* colonizing the mammalian gut. Proceedings of the National Academy of Sciences *116*, 17906-17915.

Frese, S.A., Hutton, A.A., Contreras, L.N., Shaw, C.A., Palumbo, M.C., Casaburi, G., Xu, G.*, et al.* (2017). Persistence of Supplemented *Bifidobacterium longum subsp. infantis* EVC001 in Breastfed Infants. mSphere *2*.

Garcia, A., Navarro, F., Miro, E., Villa, L., Mirelis, B., Coll, P., and Carattoli, A. (2007). Acquisition and diffusion of bla *CTX-M-9* gene by R478-IncHI2 derivative plasmids. FEMS Microbiol Lett *271*, 71-77.

Goloshchapov, O.V., Olekhnovich, E.I., Sidorenko, S.V., Moiseev, I.S., Kucher, M.A., Fedorov, D.E., Pavlenko, A.V.*, et al.* (2019). Long-term impact of fecal transplantation in healthy volunteers. BMC Microbiology *19*, 312.

Gordon, D.M., and Cowling, A. (2003). The distribution and genetic structure of *Escherichia coli* in Australian vertebrates: Host and geographic effects. Microbiology (Reading) *149*, 3575-3586.

Grozdanov, L., Raasch, C., Schulze, J., Sonnenborn, U., Gottschalk, G., Hacker, J., and Dobrindt, U. (2004). Analysis of the genome structure of the nonpathogenic probiotic *Escherichia coli* strain Nissle 1917. J Bacteriol *186*, 5432-5441.

Grozdanov, L., Zähringer, U., Blum-Oehler, G., Brade, L., Henne, A., Knirel, Y.A., Schombel, U.*, et al.* (2002). A single nucleotide exchange in the wzy gene is responsible for the semirough O6 lipopolysaccharide phenotype and serum sensitivity of *Escherichia coli* strain Nissle 1917. J Bacteriol *184*, 5912-5925.

Guenther, K., Straube, E., Pfister, W., Guenther, A., and Huebler, A. (2010). Sever Sepsis After Probiotic Treatment With *Escherichia coli* NISSLE 1917. The Pediatric Infectious Disease Journal *29*, 188-189.

Gurbatri, C.R., Radford, G.A., Vrbanac, L., Im, J., Thomas, E.M., Coker, C., Taylor, S.R.*, et al.* (2024). Engineering tumor-colonizing *E. coli* Nissle 1917 for detection and treatment of colorectal neoplasia. Nature Communications *15*, 646.

Harrison, E., and Brockhurst, M.A. (2012). Plasmid-mediated horizontal gene transfer is a coevolutionary process. Trends in Microbiology *20*, 262-267.

healthdirect (2020). Urinary tract infection (UTI). (https://www.healthdirect.gov.au/urinary-tract-infection-uti). https://www.healthdirect.gov.au/urinary-tract-infection-uti.

Helmy Yosra, A., Closs, G., Jung, K., Kathayat, D., Vlasova, A., and Rajashekara, G. (2022). Effect of Probiotic *E. coli* Nissle 1917 Supplementation on the Growth Performance, Immune Responses, Intestinal Morphology, and Gut Microbes of *Campylobacter jejuni* Infected Chickens. Infection and Immunity *90*, e00337-00322.

Hensel, M., Hinsley, A.P., Nikolaus, T., Sawers, G., and Berks, B.C. (1999). The genetic basis of tetrathionate respiration in *Salmonella typhimurium*. Molecular Microbiology *32*, 275-287.

Hudault, S., Guignot, J., and Servin, A. (2001). *Escherichia coli* strains colonising the gastrointestinal tract protect germfree mice against *Salmonella typhimurium* infection. Gut *49*, 47-55.

Jang, J., Hur, H.G., Sadowsky, M.J., Byappanahalli, M.N., Yan, T., and Ishii, S. (2017). Environmental *Escherichia coli:* ecology and public health implications-a review. J Appl Microbiol *123*, 570-581.

Janosch, D., Dubbert, S., Eiteljorge, K., Diehl, B.W.K., Sonnenborn, U., Passchier, L.V., Wassenaar, T.M.*, et al.* (2019). Anti-genotoxic and anti-mutagenic activity of *Escherichia coli* Nissle 1917 as assessed by *in vitro* tests. Benef Microbes *10*, 449-461.

Kaas, R.S., Friis, C., Ussery, D.W., and Aarestrup, F.M. (2012). Estimating variation within the genes and inferring the phylogeny of 186 sequenced diverse *Escherichia coli* genomes. BMC Genomics *13*, 577-Article.

Kan, A., Gelfat, I., Emani, S., Praveschotinunt, P., and Joshi, N.S. (2021). Plasmid Vectors for *in vivo* Selection-Free Use with the Probiotic *E. coli* Nissle 1917. ACS Synthetic Biology *10*, 94-106.

Kaper, J.B., Morris, J.G., Jr., and Levine, M.M. (1995). Cholera. Clinical microbiology reviews *8*, 48-86.

Katkowska, M., Garbacz, K., and Kusiak, A. (2021). Probiotics: Should All Patients Take Them? Microorganisms *9*.

Kay, D., Crowther, J., Stapleton, C.M., Wyer, M.D., Fewtrell, L., Edwards, A., Francis, C.A.*, et al.* (2008). Faecal indicator organism concentrations in sewage and treated effluents. Water Res *42*, 442-454.

Kruis, W., Chrubasik, S., Boehm, S., Stange, C., and Schulze, J. (2012). A double-blind placebo-controlled trial to study therapeutic effects of probiotic *Escherichia coli* Nissle 1917 in subgroups of patients with irritable bowel syndrome. International Journal of Colorectal Disease *27*, 467-474.

Lasaro, M.A., Salinger, N., Zhang, J., Wang, Y., Zhong, Z., Goulian, M., and Zhu, J. (2009). F1C Fimbriae Play an Important Role in Biofilm Formation and Intestinal Colonization by the *Escherichia coli* Commensal Strain Nissle 1917. APPLIED AND ENVIRONMENTAL MICROBIOLOGY *75*, 246-251.

Lee, H., Doak, T.G., Popodi, E., Foster, P.L., and Tang, H. (2016). Insertion sequence-caused large-scale rearrangements in the genome of *Escherichia coli*. Nucleic Acids Res *44*, 7109-7119.

Lee, Y.Y., Erdogan, A., and Rao, S.S.C. (2014). How to assess regional and whole gut transit time with wireless motility capsule. Journal of neurogastroenterology and motility *20*, 265-270.

Licht, T.R., Tolker-Nielsen, T., Holmstrom, K., Krogfelt, K.A., and Molin, S. (1999). Inhibition of *Escherichia coli* precursor-16S rRNA processing by mouse intestinal contents. Environmental Microbiology *1*, 23-32.

Lim, J.Y., Yoon, J., and Hovde, C.J. (2010). A brief overview of *Escherichia coli* O157:H7 and its plasmid O157. Journal of microbiology and biotechnology *20*, 5-14.

Lodinová-Zádniková, R., and Sonnenborn, U. (1997). Effect of preventive administration of a nonpathogenic *Escherichia coli* strain on the colonization of the intestine with microbial pathogens in newborn infants. Biol Neonate *71*, 224-232.

Lynch, S.V., and Pedersen, O. (2016). The Human Intestinal Microbiome in Health and Disease. N Engl J Med *375*, 2369-2379.

Malíčková, K., Duricová, D., Bortlík, M., Hrušková, Z., Svobodová, B., Machková, N., Komárek, V.*, et al.* (2011). Impaired deoxyribonuclease I activity in patients with inflammatory bowel diseases. Autoimmune Dis *2011*, 945861.

Manzhalii, E., Moyseyenko, V., Kondratiuk, V., Molochek, N., Falalyeyeva, T., and Kobyliak, N. (2022). Effect of a specific *Escherichia coli* Nissle 1917 strain on minimal/mild hepatic encephalopathy treatment. World Journal of Hepatology *14*, 634-646.

Matthes, H., Krummenerl, T., Giensch, M., Wolff, C., and Schulze, J. (2010). Clinical trial: probiotic treatment of acute distal ulcerative colitis with rectally administered *Escherichia coli* Nissle 1917 (EcN). BMC Complementary and Alternative Medicine *10*, 13.

Meier-Kolthoff, J.P., Hahnke, R.L., Petersen, J., Scheuner, C., Michael, V., Fiebig, A., Rohde, C.*, et al.* (2014). Complete genome sequence of DSM 30083(T), the type strain (U5/41(T)) of *Escherichia coli*, and a proposal for delineating subspecies in microbial taxonomy. Stand Genomic Sci *9*, 2.

Miller, L.E., Zimmermann, A.K., and Ouwehand, A.C. (2016). Contemporary meta-analysis of short-term probiotic consumption on gastrointestinal transit. World J Gastroenterol *22*, 5122-5131.

Moet, G.J., Jones, R.N., Biedenbach, D.J., Stilwell, M.G., and Fritsche, T.R. (2007). Contemporary causes of skin and soft tissue infections in North America, Latin America, and Europe: report from the SENTRY Antimicrobial Surveillance Program (1998-2004). Diagn Microbiol Infect Dis *57*, 7-13.

Moura de Sousa, J., Lourenço, M., and Gordo, I. (2023). Horizontal gene transfer among host-associated microbes. Cell Host & Microbe *31*, 513-527.

Mourand, G., Paboeuf, F., Grippon, P., Lucas, P., Bougeard, S., Denamur, E., and Kempf, I. (2021). Impact of *Escherichia coli* probiotic strains ED1a and Nissle 1917 on the excretion and gut carriage of extended-spectrum beta-lactamase-producing *E. coli* in pigs. Veterinary and Animal Science *14*, 100217.

Munkler, L.P., Mohamed, E.T., Vazquez-Uribe, R., Visby Nissen, V., Rugbjerg, P., Worberg, A., Woodley, J.M.*, et al.* (2024). Genetic heterogeneity of engineered *Escherichia coli* Nissle 1917 strains during scale-up simulation. Metab Eng *85*, 159-166.

Murphy, K., Curley, D., O'Callaghan, T.F., O'Shea, C.A., Dempsey, E.M., O'Toole, P.W., Ross, R.P.*, et al.* (2017). The Composition of Human Milk and Infant Faecal Microbiota Over the First Three Months of Life: A Pilot Study. Sci Rep *7*, 40597.

National Centre for Biotechnology Information (2025). NCBI datasets. Accessed: 3 October 2025. https://www.ncbi.nlm.nih.gov/datasets/genome/GCA_000714595.1/.

National Health and Medical Research Council, Australian Research Council, and Universities Australia (2018). National Statement on Ethical Conduct in Human Research 2007 (Updated 2018). (Canberra: Commonwealth of Australia, available online https://www.nhmrc.gov.au/about-us/publications/national-statement-ethical-conduct-human-research-2007-updated-2018).

Newton, L., Randall, J.A., Hunter, T., Keith, S., Symonds, T., Secrest, R.J., Komocsar, W.J.*, et al.* (2019). A qualitative study exploring the health-related quality of life and symptomatic experiences of adults and adolescents with ulcerative colitis. J Patient Rep Outcomes *3*, 66.

Norman, A., Hansen, L.H., and Sorensen, S.J. (2009). Conjugative plasmids: vessels of the communal gene pool. Philos Trans R Soc Lond B Biol Sci *364*, 2275-2289.

Nowrouzian, F.L., Wold, A.E., and Adlerberth, I. (2005). *Escherichia coli* Strains Belonging to Phylogenetic Group B2 Have Superior Capacity to Persist in the Intestinal Microflora of Infants. The Journal of Infectious Diseases *191*, 1078-1083.

NSW Goverment (2018). Shiga toxigenic *Escherichia coli* (STEC) Infection and Haemolytic Uraemic Syndrome (HUS).

OGTR (2013). Risk Analysis Framework 2013. (Office of the Gene Technology Regulator) Accessed: July 2020. http://www.ogtr.gov.au/internet/ogtr/publishing.nsf/Content/raffinal5-toc.

Olbertz, D., Proquitté, H., Patzer, L., Erler, T., Mikolajczak, A., Sadowska-Krawczenko, I., Wolff, C.*, et al.* (2023). Potential Benefit of Probiotic *E. Coli* Nissle in Term Neonates. Klin Padiatr *235*, 213-220.

Olier, M., Marcq, I., Salvador-Cartier, C., Secher, T., Dobrindt, U., Boury, M., Bacquié, V.*, et al.* (2012). Genotoxicity of *Escherichia coli* Nissle 1917 strain cannot be dissociated from its probiotic activity. Gut Microbes *3*, 501-509.

Ou, B., Yang, Y., Tham, W.L., Chen, L., Guo, J., and Zhu, G. (2016). Genetic engineering of probiotic *Escherichia coli* Nissle 1917 for clinical application. Appl Microbiol Biotechnol *100*, 8693-8699.

Petersen, A.M., Mirsepasi, H., Halkjær, S.I., Mortensen, E.M., Nordgaard-Lassen, I., and Krogfelt, K.A. (2014). Ciprofloxacin and probiotic *Escherichia coli* Nissle add-on treatment in active ulcerative colitis: A double-blind randomized placebo controlled clinical trial. Journal of Crohn's and Colitis *8*, 1498-1505.

Petersen, F., and Hubbart, J.A. (2020). Physical Factors Impacting the Survival and Occurrence of *Escherichia coli* in Secondary Habitats. Water *12*, 1796.

Pleguezuelos-Manzano, C., Puschhof, J., Rosendahl Huber, A., van Hoeck, A., Wood, H.M., Nomburg, J., Gurjao, C.*, et al.* (2020). Mutational signature in colorectal cancer caused by genotoxic *pks*(+) *E. coli*. Nature *580*, 269-273.

Poulsen, L.K., Lan, F., Kristensen, C.S., Hobolth, P., Molin, S., and Krogfelt, K.A. (1994). Spatial Distribution of *Escherichia coli* in the Mouse Large Intestine Inferred from rRNA In Situ Hybridization. Infection and Immunity *62*, 5191-5194.

Rang, C.U., Licht, T.R., Midtvedt, T., Conway, P.L., Chao, L., Krogfelt, K.A., Cohen, P.S.*, et al.* (1999). Estimation of growth rates of *Escherichia coli* BJ4 in streptomycin-treated and previously germfree mice by in situ rRNA hybridization. Clinical and Diagnostic Laboratory Immunology *6*, 434-436.

Rastegarlari, A., Gold, F., Borderon, J.C., Laugier, J., and Lafont, J. (1990). Implantation and *In-Vivo* Antagonistic Effects of Antibiotic-Susceptible *Escherichia-Coli* Strains Administered to Premature Newborns. Biology of the Neonate *58*, 73-78.

Redman, M.G., Ward, E.J., and Phillips, R.S. (2014). The efficacy and safety of probiotics in people with cancer: a systematic review. Annals of Oncology *25*, 1919-1929.

Rinninella, E., Raoul, P., Cintoni, M., Franceschi, F., Miggiano, G.A.D., Gasbarrini, A., and Mele, M.C. (2019). What is the Healthy Gut Microbiota Composition? A Changing Ecosystem across Age, Environment, Diet, and Diseases. Microorganisms *7*, 14.

Rosendahl Huber, A., Pleguezuelos-Manzano, C., Puschhof, J., Ubels, J., Boot, C., Saftien, A., Verheul, M.*, et al.* (2024). Improved detection of colibactin-induced mutations by genotoxic *E. coli* in organoids and colorectal cancer. Cancer Cell *42*, 487-496 e486.

Rudinsky, A.J., Harrison, A., Shi, B., Hardison, R., Prinster, T., Huang, S., Lee, S.*, et al.* (2023). The use of *Escherichia coli* strain Nissle 1917 shows promise for improving gastrointestinal and urinary health in dogs. Am J Vet Res *84*.

Russell, A.D. (1990). Bacterial spores and chemical sporicidal agents. Clin Microbiol Rev *3*, 99-119.

Russell, J.B., and Jarvis, G.N. (2001). Practical mechanisms for interrupting the oral-fecal lifecycle of *Escherichia coli*. Journal of Molecular Microbiology and Biotechnology *3*, 265-272.

Russo, T.A., and Johnson, J.R. (2003). Medical and economic impact of extraintestinal infections due to *Escherichia coli*: focus on an increasingly important endemic problem. Microbes and Infection *5*, 449-456.

Rutala, W.A., Weber, D.J., and Healthcare Infection Control Practices Advisory Committee (2008). Guideline for Disinfection and Sterilization in Healthcare Facilities. (United States: Centres for Disease Control and Prevention).

Sassone-Corsi, M., Nuccio, S.P., Liu, H., Hernandez, D., Vu, C.T., Takahashi, A.A., Edwards, R.A., *et al.* (2016). Microcins mediate competition among *Enterobacteriaceae* in the inflamed gut. Nature *540*, 280-283.

Savageau, M.A. (1983). *Escherichia-Coli* Habitats Cell Types and Molecular Mechanisms of Gene Control. American Naturalist *122*, 732-744.

Schamberger, G.P., Phillips, R.L., Jacobs, J.L., and Diez-Gonzalez, F. (2004). Reduction of *Escherichia coli* O157:H7 populations in cattle by addition of colicin E7-producing *E. coli* to feed. Applied and Environmental Microbiology *70*, 6053-6060.

Sedaghat, A., Karimi Torshizi, M.A., and Soleimani, M.R. (2025). Effects of *Escherichia coli* Nissle 1917 on immunity, blood constituents, antioxidant capacity, egg quality and performance in laying Japanese quail. Poultry Science *104*, 104741.

Sender, R., Fuchs, S., and Milo, R. (2016). Revised Estimates for the Number of Human and Bacteria Cells in the Body. PLoS Biol *14*, e1002533.

Shanks, O.C., Newton, R.J., Kelty, C.A., Huse, S.M., Sogin, M.L., and McLellan, S.L. (2013). Comparison of the microbial community structures of untreated wastewaters from different geographic locales. Appl Environ Microbiol *79*, 2906-2913.

Sonnenborn, U. (2016). *Escherichia coli* strain Nissle 1917—from bench to bedside and back: history of a special *Escherichia coli* strain with probiotic properties. FEMS Microbiology Letters *363*.

Sonnenborn, U., and Schulze, J. (2009). The non-pathogenic *Escherichia coli* strain Nissle 1917 – features of a versatile probiotic. Microbial Ecology in Health and Disease *21*, 122-158.

Sørensen, S.J., Bailey, M., Hansen, L.H., Kroer, N., and Wuertz, S. (2005). Studying plasmid horizontal transfer *in situ*: a critical review. Nature Reviews Microbiology *3*, 700-710.

Sousa, A., Bourgard, C., Wahl, L.M., and Gordo, I. (2013). Rates of transposition in *Escherichia coli*. Biol Lett *9*, 20130838.

Standards Australia/New Zealand (2022). *AS/NZS 2243.3:2022* Safety in laboratories, Part 3: Microbiological safety and containment. In Edition AS/NZS 22433:2022 Standards Australia/New Zealand.

Swirkosz, G., Szczygiel, A., Logon, K., Wrzesniewska, M., and Gomulka, K. (2023). The Role of the Microbiome in the Pathogenesis and Treatment of Ulcerative Colitis-A Literature Review. Biomedicines *11*.

Tenaillon, O., Skurnik, D., Picard, B., and Denamur, E. (2010). The population genetics of commensal *Escherichia coli*. Nature Reviews Microbiology *8*, 207-217.

The Queensland Government (2025). Guideline: Clinical and related waste, T.S.a.I. Department of the Environment, ed. (Queensland).

Toze, S., Hodgers, L., Matthews, B., Stratton, H., Ahmed, W., Collins, S., Schoeder, S*., et al.* (2012). Presence and removal of enteric microorganisms in South East Queensland wastewater treatment plants. . (Urban Water Security Research Alliance; 2012. http://hdl.handle.net/102.100.100/100932?index=1).

Vally, H., Hall, G., Dyda, A., Raupach, J., Knope, K., Combs, B., and Desmarchelier, P. (2012). Epidemiology of Shiga toxin producing *Escherichia coli* in Australia, 2000-2010. BMC Public Health *12*, 63.

van Elsas, J.D., Semenov, A.V., Costa, R., and Trevors, J.T. (2011). Survival of *Escherichia coli* in the environment: fundamental and public health aspects. The ISME Journal *5*, 173-183.

Verdugo-Meza, A., Gill, S.K., Godovannyi, A., Adur, M.K., Barnett, J.A., Estaki, M., Ye, J*., et al.* (2024). Bio-engineering a common probiotic to exploit colonic inflammation promotes reliable efficacy in translational models of colitis. bioRxiv, 2024.2010.2008.617317.

Vila, J., Sáez-López, E., Johnson, J.R., Römling, U., Dobrindt, U., Cantón, R., Giske, C.G*., et al.* (2016). *Escherichia coli*: an old friend with new tidings. FEMS Microbiol Rev *40*, 437-463.

Vollaard, E., and Clasener, H. (1994). Mini-review: Colonization resistance. Antimicrobial Agents and Chemotherapy *38*, 409-414.

von Buenau, R., Jaekel, L., Schubotz, E., Schwarz, S., Stroff, T., and Krueger, M. (2005). *Escherichia coli* Strain Nissle 1917: Significant Reduction of Neonatal Calf Diarrhea. Journal of Dairy Science *88*, 317-323.

Wang, Q., Wei, S., Silva, A.F., and Madsen, J.S. (2023). Cooperative antibiotic resistance facilitates horizontal gene transfer. The ISME Journal *17*, 846-854.

Wassenaar, T.M. (2016). Insights from 100 Years of Research with Probiotic *E. Coli*. European journal of microbiology & immunology *6*, 147-161.

Winter, S.E., and Bäumler, A.J. (2011). A breathtaking feat: to compete with the gut microbiota, *Salmonella* drives its host to provide a respiratory electron acceptor. Gut Microbes *2*, 58-60.

Wu, S., Zhang, Q., Cong, G., Xiao, Y., Shen, Y., Zhang, S., Zhao, W*., et al.* (2023). Probiotic *Escherichia coli* Nissle 1917 protect chicks from damage caused by *Salmonella enterica* serovar Enteritidis colonization. Anim Nutr *14*, 450-460.

Yang, I., Corwin, E.J., Brennan, P.A., Jordan, S., Murphy, J.R., and Dunlop, A. (2016). The Infant Microbiome: Implications for Infant Health and Neurocognitive Development. Nursing research *65*, 76-88.

Yin, Q., da Silva, A.C., Zorrilla, F., Almeida, A.S., Patil, K.R., and Almeida, A. (2025). Ecological dynamics of *Enterobacteriaceae* in the human gut microbiome across global populations. Nature Microbiology *10*, 541-553.

Zhang, T., Shao, M.F., and Ye, L. (2012). 454 pyrosequencing reveals bacterial diversity of activated sludge from 14 sewage treatment plants. ISME J *6*, 1137-1147.

Zmora, N., Zilberman-Schapira, G., Suez, J., Mor, U., Dori-Bachash, M., Bashiardes, S., Kotler, E*., et al.* (2018). Personalized Gut Mucosal Colonization Resistance to Empiric Probiotics Is Associated with Unique Host and Microbiome Features. Cell *174*, 1388-1405.e1321.

Zuo, H.-J., Xie, Z.-M., Zhang, W.-W., Li, Y.-R., Wang, W., Ding, X.-B., and Pei, X.-F. (2011). Gut bacteria alteration in obese people and its relationship with gene polymorphism. World journal of gastroenterology *17*, 1076-1081.