Environmental Protection Authority
Te Mana Rauhī Taiao

APPLICATION FORM
Containment

To obtain approval for new organisms in containment

Send to Environmental Protection Authority preferably by email (neworganisms@epa.govt.nz) or alternatively by post (Private Bag 63002, Wellington 6140)
Payment must accompany final application; see our fees and charges schedule for details.

Application Number

APP204024

Date

4/9/20

Completing this application form

1. This form has been approved under section 40 of the Hazardous Substances and New Organisms (HSNO) Act 1996. It only covers importing, development (production, fermentation or regeneration) or field test of any new organism (including genetically modified organisms (GMOs)) in containment. If you wish to make an application for another type of approval or for another use (such as an emergency, special emergency or release), a different form will have to be used. All forms are available on our website.
2. If your application is for a project approval for low-risk GMOs, please use the Containment – GMO Project application form. Low risk genetic modification is defined in the HSNO (Low Risk Genetic Modification) Regulations: http://www.legislation.govt.nz/regulation/public/2003/0152/latest/DLM195215.html.
3. It is recommended that you contact an Advisor at the Environmental Protection Authority (EPA) as early in the application process as possible. An Advisor can assist you with any questions you have during the preparation of your application including providing advice on any consultation requirements.
4. Unless otherwise indicated, all sections of this form must be completed for the application to be formally received and assessed. If a section is not relevant to your application, please provide a comprehensive explanation why this does not apply. If you choose not to provide the specific information, you will need to apply for a waiver under section 59(3)(a)(ii) of the HSNO Act. This can be done by completing the section on the last page of this form.
5. Any extra material that does not fit in the application form must be clearly labelled, cross-referenced, and included with the application form when it is submitted.
6. Please add extra rows/tables where needed.
7. You must sign the final form (the EPA will accept electronically signed forms) and pay the application fee (including GST) unless you are already an approved EPA customer. To be recognised by the EPA as an “approved customer”, you must have submitted more than one application per month over the preceding six months, and have no history of delay in making payments, at the time of presenting an application.
8. Information about application fees is available on the EPA website.
9. All application communications from the EPA will be provided electronically, unless you specifically request otherwise.

Commercially sensitive information

10. Commercially sensitive information must be included in an appendix to this form and be identified as confidential. If you consider any information to be commercially sensitive, please show this in the relevant section of this form and cross reference to where that information is located in the confidential appendix.
11. Any information you supply to the EPA prior to formal lodgement of your application will not be publicly released. Following formal lodgement of your application any information in the body of this application form and any non-confidential appendices will become publicly available.
12. Once you have formally lodged your application with the EPA, any information you have supplied to the EPA about your application is subject to the Official Information Act 1982 (OIA). If a request is made for the release of information that you consider to be confidential, your view will be considered in a manner consistent with the OIA and with section 57 of the HSNO Act. You may be required to provide further justification for your claim of confidentiality.

Definitions

Containment	Restricting an organism or substance to a secure location or facility to prevent escape. In respect to genetically modified organisms, this includes field testing and large scale fermentation
Controls	Any obligation or restrictions imposed on any new organism, or any person in relation to any new organism, by the HSNO Act or any other Act or any regulations, rules, codes, or other documents made in accordance with the provisions of the HSNO Act or any other Act for the purposes of controlling the adverse effects of that organism on people or the environment
Genetically Modified Organism (GMO)	Any organism in which any of the genes or other genetic material: • Have been modified by *in vitro* techniques, or • Are inherited or otherwise derived, through any number of replications, from any genes or other genetic material which has been modified by *in vitro* techniques
New Organism	A new organism is an organism that is any of the following: • An organism belonging to a species that was not present in New Zealand immediately before 29 July 1998; • An organism belonging to a species, subspecies, infrasubspecies, variety, strain, or cultivar prescribed as a risk species, where that organism was not present in New Zealand at the time of promulgation of the relevant regulation; • An organism for which a containment approval has been given under the HSNO Act; • An organism for which a conditional release approval has been given under the HSNO Act; • A qualifying organism approved for release with controls under the HSNO Act; • A genetically modified organism; • An organism belonging to a species, subspecies, infrasubspecies, variety, strain, or cultivar that has been eradicated from New Zealand;

	• An organism present in New Zealand before 29 July 1998 in contravention of the Animals Act 1967 or the Plants Act 1970. This does not apply to the organism known as rabbit haemorrhagic disease virus, or rabbit calicivirus A new organism does not cease to be a new organism because: • It is subject to a conditional release approval; or • It is a qualifying organism approved for release with controls; or • It is an incidentally imported new organism
Project	An individual or collaborative endeavour that is planned to achieve a particular aim or research goal

1. Applicant details

1.1. Applicant

Company Name: (if applicable) AgResearch

Contact Name: Mallory Crookenden

Job Title: Post-Doctoral Scientist

Physical Address: Hopkirk Research Institute, Massey University, Manawatū Campus

Postal Address (provide only if not the same as the physical)**:** Hopkirk Reserch Institute, Cnr Library Rd and University Ave, Massey University, Palmerston North, Manawatū, New Zealand 4410

Phone (office and/or mobile): +6463518777

Fax:

Email: mallory.crookenden@agresearch.co.nz

1.2. New Zealand agent or consultant (if applicable)

Company Name:

Contact Name:

Job Title:

Physical Address:

Postal Address (provide only if not the same as the physical)**:**

Phone (office and/or mobile):

Fax:

Email:

2. Information about the application

2.1. Type of containment activity

Tick the box(es) that best describe your application

Application type	Type of new organism
Import into containment	☐ GMO
	☐ Non-GMO
Develop in containment i.e. regeneration, fermentation or genetic modification	☐ GMO
	☒ Non-GMO
Field test in containment	☐ GMO
	☐ Non-GMO

2.2. Brief application description

Approximately 30 words about what you are applying to do

The applicant intends to undertake research towards the development of a diagnostic test for *Mycoplasma bovis*. To do this, we require the ability to propagate and work with New Zealand-sourced strains of *Mycoplasma bovis* in the Hopkirk Research Institute PC3 containment facility.

2.3. Summary of application

Provide a plain English, non-technical description of what you are applying to do and why you want to do it

We are receiving MPI funding to develop a new test for diagnosing *Mycoplasma bovis* that can be used in the absence of bacterial shedding, or when the antibody response is poor. The test will involve identifying bacterial protein signatures within circulating nanoparticles known as exosomes. The concept being that exosomes will be 'shed' from cells that are infected with *Mycoplasma bovis* and contain proteins that are specific to infected animals, even when the bacterium is not exposed to the immune system. Exosomes are used for inter-cellular signalling and, therefore, provide an indication of the health status of their cell of origin and can be recovered from a number of different sample types. This means they are readily accessible and likely contain cargo that are specific depending on the infection-status of an animal.

Test development includes a non-targeted proteomic approach to identify exosomal proteins that are specific to *Mycoplasma bovis* infection. Exosomes will be isolated, initially from cell culture media from cells that have been 'infected' with *Mycoplasma bovis in vitro*. The goal of this is to discover an exosome protein-cargo signature of *Mycoplasma bovis* infection, which will be validated from exosomes collected from a variety of biological samples from healthy and infected animals (provided by MPI).

Live *Mycoplasma bovis* will be transferred from MPI Wallaceville to the Hopkirk Research Institute and cultured in the PC3 facility using sterile technique and cell culture methods. Prior to removal from PC3 for subsequent analysis under PC2 exosome preparations will be sterilised. Various sterilisation methods will be tested and validated to ensure sterility of all samples taken out of the PC3 lab (see section 4.2).

2.4. Background and aims of application

This section is intended to put the new organism(s) in perspective of the wider activities that they will be used in. You may use more technical language but all technical words must be included in a glossary

The project will have two phases: i) Identification of a protein profile from exosomal cargo that can be used as a signature for *Mycoplasma bovis* infection status using non-targeted proteomics. ii) development of a diagnostic test using targeted proteins from the protein signature identified in (i).

i) Identifying a protein signature of *Mycoplasma bovis* infection

- Cells will be cultured *in vitro* and co-cultured with live *Mycoplasma bovis* to produce exosomes into the cell culture supernatant in a PC3 containment laboratory.
- Exosomes will be isolated from the supernatant and the isolation method will be validated. This will also be undertaken in the PC3 containment facility.
- Exosome preparations will be sterilised and then removed from PC3 to be further processed and analysed in a PC2 laboratory.
- Exosomal vesicles are characterized by membrane proteins that can be used to verify isolation. For example, the presence of proteins CD63, TSG101, and CD81 will be used to verify exosomes.
  N.B. The above validation will be conducted at PC2 level containment on isolated exosomes/exosomal protein.

Non-targeted protein analysis: Proteomics on exosomes isolated from infected and non-infected animals using Mass Spectrometry (LC-MS/MS). This will involve discovery of *Mycoplasma bovis*-specific proteins that are unique to infected individuals.

Targeted protein analysis. The identification of *Mycoplasma bovis*-specific proteins will be used for targeting protein analysis in a variety of biological fluids. The protein signature will need to be validated in exosomes from several sources to ensure the robustness of the test.

ii) Development of a diagnostic test

The protein signature identified in (i) will be used to develop a targeted protein diagnostic test, this will be done in collaboration with the diagnostic laboratory.

3. Information about the new organism(s)

3.1. Name of organism

Identify the organism as fully as possible

Non-GMOs - Provide a taxonomic description of the new organism(s).

GMOs – Provide a taxonomic description of the host organism(s) and describe the genetic modification.

Both -

- Describe the biology and main features of the organism including if it has inseparable organisms.
- Describe if the organism has affinities (e.g. close taxonomic relationships) with other organisms in New Zealand.
- Could the organism form an undesirable self-sustaining population? If not, why not?
  How easily could the new organism be recovered or eradicated if it established an undesirable self-sustaining population? -

Non-GMO:

Scientific Name: *Mycoplasma bovis*

Synonyms: *Mycoplasma agalactiae* subsp *bovis, Mycoplasma bovimastitidis*

Common Name(s): *Mycoplasma bovis*

Classification:

Domain: Bacteria

Phylum: Tenericutes

Class: Mollicutes

Order: Mycoplasmatales

Family: Mycoplasmataceae

Genus: *Mycoplasma*

Species: *Mycoplasma bovis*

Mycoplasma are a subset of bacteria, notable for their absence of a cell wall and very small size (less than a micron in diameter). The lack of a solid cell wall gives them the ability to change shape, and to change their outer membrane rapidly. They are neither rods nor cocci. Their DNA length is amongst the smallest of self-replicating organisms and they have an unusually low guanidine-cytosine ratio of their nucleotides.

Mycoplasma bovis causes bovine mycoplasmosis, an infection that leads to a variety of clinical manifestations, mostly of a chronic nature, including bronchopneumonia, otitis, mastitis, genital disorders, arthritis, meningitis or keratoconjunctivitis. While primarily an organism associated with cattle, studies have isolated *Mycoplasma bovis* from goats, chickens, humans and pigs, but often in these situations there is an association with another underlying condition.

In July 2017, *Mycoplasma bovis* was found in cattle in the Oamaru area of the South Island. The organism has now been found in many other parts of the North and South Islands. There are other Mycoplasma in NZ, but no interaction between *Mycoplasma bovis* and other Mycoplasma species. Therefore, this organism is already undesired and self-sustaining, which is why we're doing what we're doing. Currently, there is a nationwide programme to eradicate *Mycoplasma bovis* from New Zealand farms based on current diagnostic tests and slaughter of infected animals.

Mycoplasma bovis is an Incidentally Imported New Organism (IINO). As such, the proposed activity involving the "deliberate isolation, aggregation, multiplication, or other use of the organism" constitutes an activity to 'develop' the IINO under the HSNO Act and therefore requires an approval from the EPA.

3.2. Regulatory status of the organism

Is the organism that is the subject of this application also the subject of:

An innovative medicine application as defined in section 23A of the Medicines Act 1981?

☐ Yes ☒ No

An innovative agricultural compound application as defined in Part 6 of the Agricultural Compounds and Veterinary Medicines Act 1997?

☐ Yes ☒ No

4. Information about the containment

4.1. For field tests: The nature and method of the field test

Describe the nature and method of the field test and the experimental procedures to be used

N/A

4.2. Proposed containment of the new organism(s) (physical and operational)

Describe how you propose to contain the new organism(s) after taking into account its ability to escape from containment (i.e. the possible pathways for escape)

Laboratory work culturing the IINO i.e., viable *Mycoplasma bovis*, will be done in a PC3 lab and a PC2 laboratory will be used for work with non-viable preparations (i.e., isolated exosomes).

Containment information for PC3 laboratory where work with this new organism will be undertaken:

- Hopkirk Institute PC3 lab is situated on the second floor of the Hopkirk Research Institute and consists of three PC3 laboratories, each comprising a suite of rooms, each individual suite entered via an airlock from a restricted PC2 corridor (corridor 2.07). Rooms 2.08, 2.09, 2.10, 2.11, 2.12, 2.13, comprise the AgResearch Food Assurance & Meat Quality PC3 Laboratory and AgResearch TB Diagnostics Laboratory and rooms 2.14, 2.15, 2.16 and 2.17 comprise the AgResearch Animal Health PC3 Laboratory, which is where this work will be undertaken.
- The Hopkirk Research Institute's PC3 laboratories meet the minimum requirements for PC3 as identified in AS/NZS 2243.3: 2002, Sections 4.9.2 and 4.9.3.

Operational points related to the handling of samples and *Mycoplasma bovis* strains in the Hopkirk Institute's PC3 containment facility are as follows:

- Samples not sealed within a primary container are handled in Class II biosafety cabinets.
- Samples that are in storage will be clearly labelled as part of the *M. bovis* surveillance programme and only handled by trained and approved staff.
- Centrifuges fitted with either sealed rotors or sealed buckets shall be used.

o Laboratory wastes shall be rendered safe, preferably be decontamination in a pressure steam steriliser either before dispatch from the laboratory or will be sent direct for treatment/decontamination to a suitable supplier.

o Entry to the laboratory during laboratory testing is restricted to fully-trained and authorised laboratory personnel.

o Outer clothing and personal effects shall not be taken into the laboratory.

o Protective clothing will completely cover all normal clothing on arms and the front of the operator (no front opening white clothes).

o Protective clothing used within the laboratory where samples are handled shall not be worn outside the facility and shall be decontaminated by pressure steam sterilisation prior to laundering or disposal.

o Protective clothing shall be removed in a predetermined appropriate order and immediately sealed in a bag for later decontamination.

o Measures shall be taken to ensure no microbiological contamination is removed from the laboratory on footwear. A change of footwear is recommended.

o No-one shall enter the laboratory for cleaning, servicing equipment, repairs or other activities before the relevant, potentially contaminated laboratory surfaces and equipment have been decontaminated.

Chemical sterilisation or heat inactivation will be used as per established protocol for removing items from a PC3 laboratory:

o All dry waste (e.g., masks, paper, gloves) used within a Class II cabinet shall be collected in a plastic bag within a container in the Class II cabinet. When full, the top of the bag should be folded over to create a loose seal, then placed within an autoclave bag labelled "Biological Hazard" prior to removal from the Class II cabinet

o Liquid and plastic waste will be disinfected using steri-GENE Clear and UV treated in the Class II cabinet. The containers must then be covered with at least 2 layers of foil prior to removal from the Class II cabinet.

o General laboratory waste shall be collected in biohazard bags and sealed using tape. The exterior of the bags shall be sprayed thoroughly with 2% Steri-GENE Clear disinfectant and held in the closed waste bins provided in each laboratory.

o The waste bin shall then be taken out of the laboratory and the autoclave bags placed in the sealed, plastic waste bin in the Restricted Corridor 2.07 to await autoclaving. All waste should be autoclaved as soon as possible after bags/containers are placed in the bin – preferably the bags should go straight into the autoclave.

o Autoclave waste will be disposed of as per waste disposal protocols of the Hopkirk Research Institute.

5. Māori engagement

Discuss any engagement or consultation with Māori undertaken and summarise the outcomes. Please refer to the EPA policy 'Engaging with Māori for applications to the EPA' on our website (www.epa.govt.nz) or contact the EPA for advice.

We have received feedback from the EPA Māori advisory team (KKT) who do not have any concerns.

6. Risks, costs and benefits

Provide information of the risks, costs and benefits of the new organism(s).

These are the positive and adverse effects referred to in the HSNO Act. It is easier to regard risks and costs as being adverse (or negative) and benefits as being positive. In considering risks, cost and benefits, it is important to look at both the likelihood of occurrence (probability) and the potential magnitude of the consequences, and to look at distribution effects (who bears the costs, benefits and risks).

Consider the adverse or positive effects in the context of this application on the environment (e.g. could the organism cause any significant displacement of any native species within its natural habitat, cause any significant deterioration of natural habitats or cause significant adverse effect to New Zealand's inherent genetic diversity, or is the organism likely to cause disease, be parasitic, or become a vector for animal or plant disease?), human health and safety, the relationship of Māori to the environment, the principles of the Treaty of Waitangi, society and the community, the market economy and New Zealand's international obligations.

You must fully complete this section referencing supporting material. You will need to provide a description of where the information in the application has been sourced from, e.g. from in-house research, independent research, technical literature, community or other consultation, and provide that information with this application.

The bacterium is already an unwanted organism in NZ (IINO) with infection of multiple cattle herds across the country. The work proposed here is part of a research program contributing to the eradication effort that is funded by MPI.

Successful development of the novel exosome diagnostic test will enable better testing, particularly allow detection of animals with a "silent" *Mycoplasma bovis* infection, which

will aid in the eradication program and consequently provide a cost benefit to New Zealand.

7. Alternative methods and potential effects from the transfer of genetic elements

This section is for developments of GMOs that take place outdoors and field tests of GMOs only

- Discuss if there are alternative methods of achieving the research objective.
- Discuss whether there could be effects resulting from the transfer of genetic elements to other organisms in or around the site of the development or field test.

N/A – for development of our diagnostic test we need the exosomes produced by infected cells, which can fortunately be done *in vitro* using cell culture in PC3 containment.

8. Pathway determination and rapid assessment

This section is for the imports of GMOs only

Under section 42B of the HSNO Act your application may be eligible for a rapid assessment. The pathway for your application will be determined after its formal receipt, based on the data provided in this application form. If you would like your application to be considered for rapid assessment (as per the criteria below), we require you to complete this section.

8.1. Discuss whether the GMO(s) to be imported fulfil the criteria

The criteria are:

- The host organism(s) are Category 1 or 2 host organisms as per the HSNO (Low Risk Genetic Modification) Regulations
- The genetic modifications are Category A or B modifications as per the HSNO (Low Risk Genetic Modification) Regulations and the modifications are not listed in the Schedule of these Regulations
- The minimum containment of the GMO(s) will be as per the HSNO (Low Risk Genetic Modification) Regulations (PC1 or PC2 as per AS/NZS2243.3:2002)

N/A – the organism is not a GMO.

9. Other information

Add here any further information you wish to include in this application including if there are any ethical considerations that you are aware of in relation to your application.

This project will be done in collaboration with MPI.

We have had a section 52 approval in the past (see appendix) and are currently seeking a section 53 approval under the biosecurity act.

10. Checklist

This checklist is to be completed by the applicant

Application		**Comments/justifications**
All sections of the application form completed or you have requested an information waiver under section 59 of the HSNO Act	☒ Yes ☐ No (If No, please discuss with an Advisor to enable your application to be further processed)	
Confidential data as part of a separate, identified appendix	☒ Yes ☐ No	A section 52 CTO approval is included as a confidential appendix. A section 53 permission will also be required in order to propagate *M. bovis*, this is currently in the approval process with MPI.
Supplementary optional information attached:		
• Copies of additional references	☐ Yes ☒ No	
• Relevant correspondence	☐ Yes ☒ No	
Administration		
Are you an approved EPA customer?	☐ Yes ☒ No If Yes are you an: Applicant: ☐ Agent: ☐	
If you are not an approved customer, payment of fee will be by: • Direct credit made to the EPA bank account (preferred method of payment) Date of direct credit: • Cheque for application fee enclosed	☐ Yes ☐ No ☒ Payment to follow ☐ Yes ☐ No ☐ Payment to follow	
Electronic, signed copy of application e-mailed to the EPA	☐ Yes	

Signature of applicant or person authorised to sign on behalf of applicant

☐ I am making this application, or am authorised to sign on behalf of the applicant or applicant organisation.

☒ I have completed this application to the best of my ability and, as far as I am aware, the information I have provided in this application form is correct.

4/9/20

Signature **Date**

Request for information waiver under section 59 of the HSNO Act

☐ I request for the Authority to waive any legislative information requirements (i.e. concerning the information that has been supplied in my application) that my application does not meet (tick if applicable).

Please list below which section(s) of this form are relevant to the information waiver request:

Appendices and referenced material (if any) and glossary (if required)

A previously approved section 52 CTO approval is included as a confidential appendix below.

Section 52 application_Koru Di