

**UNIVERSITY OF
AUCKLAND**
Waipapa Taumata Rau
NEW ZEALAND

Biological Risk Management and Containment

LABORATORY USERS' QUICK REFERENCE GUIDE



INTRODUCTION

This quick reference guide is designed to provide you with step-by-step guidance on all the basic procedures you will need to conduct your work in the laboratory safely and efficiently.

Each section below gives you information on a process or procedure that needs to be followed to meet the University's standards of professional practice in our containment and transitional facilities. Please note: Terms that appear in bold type are defined in the glossary at the back of this guide.

More detailed guidelines and training aids are available on the Biomedical Risk Management & Containment website (see QR code for link)



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LEARN IT

What you need to know

HSNO approvals

Under the Hazardous Substances and New Organisms (HSNO) Act (1996), all staff and students are required to obtain prior HNSO approval from the Ministry for Primary Industries (MPI) before starting any development or importation of **genetically modified organisms** (GMOs).

Before starting any work with GMOs, you must consult with your **principal investigator** (PI), who holds the research group's HSNO approval.

If this approval does not cover the work you are thinking about, talk to the PI or **designated laboratory person** (DLP), who may be able to apply for an amendment.

You must comply with all controls imposed as a condition of the HSNO approval (including additional controls imposed as a condition of approval), and you must conduct all work involving GMOs within the confines of a MPI-approved containment facility.

Emergency response

- Are you familiar with emergency evacuation procedures?
- Do you know where the fire exits are?
- Do you know where the fire extinguisher is?
- Do you know who the first aider is?
- Do you know where the safety shower is?
- Do you know where the eyewash station is?

Safety Shower



- Do you know where the spill kits are kept?

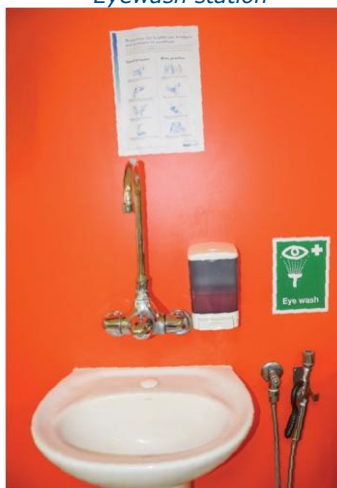
Before you start work

- If you have long hair, please tie it back
- Do not wear dangling jewellery
- Make sure your lab coat is buttoned up
- If you have cuts or sores on your hands, report to your supervisor so that they can be covered with a dressing
- Are you wearing appropriate footwear? No sandals, jandals, ballet flats, Crocs or open-toed shoes please
- Have you got appropriate PPE (safety glasses, gloves, mask, ventilator) for the work you are doing?
- Do not use digital devices (cellphones, laptops, tablets) in the lab
- Leave your personal belongings in lockers outside the lab
- To control the risk of infection, no eating, drinking or applying cosmetics in the lab
- Make sure your benchtop and surrounding areas are clean and tidy

At the end of your session

- Ensure all your materials are labelled with your name and experiment number/research project name
- Place cultures for incubation in labelled plastic containers
- Place all sharps in designated container
- Dispose of contaminated materials strictly in accordance with the instructions in this guide
- Decontaminate bench surfaces strictly in accordance with the instructions in this guide
- Always wash your hands before leaving the lab: this is an important safety measure

Eyewash station



CLEAN IT/KILL IT Chemical decontamination

A. Liquid biohazardous waste

Because biohazardous waste presents a risk to personal health and safety and often to New Zealand's biosafety, all waste from PC1 and PC2 laboratories must be sterilised or autoclaved* before disposal. We strongly recommend using an autoclave for the decontamination of high organic load** liquid wastes. However, it is acceptable to use approved chemical disinfectants instead of an autoclave:

- To decontaminate small volumes of liquid bacterial and GM bacterial cultures
- To decontaminate low organic load liquid waste
- To decontaminate virus cultures if the chemical agent has been demonstrated to be viricidal
- To decontaminate solutions containing fungi

Note: Holding times must be observed to ensure the chemical disinfectant performs adequately.

* See page 8 for information about autoclaving.

** High organic load waste = cell cultures $>10^5$ cells/mL or blood/body fluids that have high organic load

Approved decontamination agent	Final concentration or dilution	Holding time
Sodium hypochlorite	0.5%	60 minutes
Trigene/ Chemgene	1:50 of concentrate	60 minutes
Virkon	1%	60 minutes
Accel Prevail	1:40 of concentrate	20 minutes

Notes:

1. Hypochlorite solutions will lose efficacy rapidly in the presence of organic material. Therefore sodium hypochlorite is not suitable for decontaminating large volumes of liquid biohazardous waste with a high organic load.
2. Handle sodium hypochlorite with care as it is corrosive and will damage stainless steel surfaces and clothing. When you discharge solutions containing hypochlorite into the sewer, rinse stainless steel sinks with copious amounts of water afterwards.

B. Benchtop decontamination

It is equally important to decontaminate your bench area several times a day, taking special care when you have finished your work for the day and are about to leave the lab. We recommend using the following approved decontamination agents.

Approved decontamination agent	Final dilution	Holding time
Trigene/ Chemgene	1:100	5 minutes
Accel Prevail	1:40 of concentrate	5 minutes
Tristel Jet	N/A; cannot be diluted	1 minutes

Notes:

1. For blood, heavily soiled areas, fungi or where Risk Group 2 pathogens are involved, higher concentrated solutions must be used. Please refer to Benchtop Decontamination Guidelines for more detail.
2. Ethanol is a cleaning and sanitising agent, not a decontamination agent.

C. Stable shelf life of dilutions

Decontamination agent	Working life
Hypochlorite	1 week
Trigene Advance	6 months
Accel Prevail	1 month
Chemgene HLD4	3 months
Tristel Jet	N/A; cannot be diluted

D. Storage of dilutions

All dilutions must be stored in bottles labelled with:

- The name of the decontamination agent
- The dilution or concentration of the decontamination agent
- The expiry date of the dilution

Note: Diluted working solutions of hypochlorite must not be stored in glass bottles as exposure to light hastens hypochlorite decay.

CLEAN-UP FOR MICROBIOLOGICAL SPILLS

Spills inside biological safety cabinets (BSCs)

Small spills (droplets up to 1mL)

1. Leave the BSC on.
2. Wipe with disinfectant-soaked absorbent material, or flood with a suitable disinfectant solution and leave for at least 10 minutes.

Larger spills

1. Leave the cabinet on.
2. Place absorbent material wetted with an approved disinfectant and leave for at least 10 minutes.
3. Disinfect and remove protective gloves in the cabinet.
4. Remove any contaminated clothing for decontamination.
5. Wash hands and arms.
6. Replace with clean gloves and protective clothing for carrying out the remainder of the clean-up.
7. After initial disinfection, remove any sharp objects with forceps and discard as contaminated sharps.
8. Remove excess fluid with absorbent material and discard into a container for decontamination.
9. Discard culture bottles, petri dishes and solid material associated with the spill into the same container.
10. Decontaminate cultures, media and disposable materials adjacent to the spill.
11. Wipe down the cabinet floor, cabinet work zone and remaining items of equipment with fresh disinfectant solution. For Class II cabinets, disinfect both sides of the front grille and work floor within the cabinet.
12. Check that the spillage has not contaminated the sump. If the sump is contaminated, add sufficient disinfectant solution to completely cover the sump floor. If the spill is large, use sufficient disinfectant solution to dilute and inactivate the infectious material.
13. Consider whether the cabinet should be decontaminated before further use.
14. Complete an accident/incident report.



Spills outside biological safety cabinets

1. Contain the spill whenever possible.
2. Move away from the spill.
3. Remove the laboratory gown and any other garment suspected of being contaminated, and place in a biohazard bag for decontamination.
4. Warn others to keep out of the area of the spill.
5. If contamination of the lab user is superficial, wash exposed skin with soap and water or an approved ethanolbased handwash such as Sterigel, and put on a clean laboratory gown. Use an eye wash station if the eyes or face have been exposed.
6. Leave the area and place a temporary sign with DO NOT ENTER on the door.
7. Report the spill to the floor or sector manager, technical manager or technical team leader.

Disposal of contaminated waste following spill clean-up

- Normal laboratory waste disposal procedures should be used for spill clean-up material.
- Spill clean-up material containing disinfectants should not normally be autoclaved as they can damage the autoclave or produce toxic vapours.

Centrifuge spills

1. For sealed rotors or buckets that can withstand high temperatures, autoclave intact at 121°C for a minimum of 15 minutes.
2. For rotors and buckets not able to withstand high temperatures: Where breakage or spillage is observed, allow 30 minutes for aerosols to settle. Place the rotor or bucket in an approved non-corrosive disinfectant solution.
3. If the disinfectant is corrosive, wipe internal surfaces with water or detergent at the end of the contact time. Avoid using glass centrifuge tubes, as they are easily broken. If a glass centrifuge tube has broken, it is important to clean up properly. Remove larger pieces of broken glass to the sharps container with forceps and use material such as cotton wool moistened with disinfectant to pick up the smaller pieces.
4. Wipe internal surfaces of the centrifuge bowl with disinfectant.

AUTOCLAVING

What are autoclaves used for?

The autoclaves approved for use in University of Auckland containment laboratories employ pressurised steam to sterilise biohazardous waste.

Sterilisation of GM and containment waste

Steam sterilisation must be carefully and regularly monitored to ensure that all parts of the load achieve sterilising conditions consistently. The University is required to verify and demonstrate the results to the community and regulators.

Consequently GM and containment waste can only be steam sterilised using autoclaves that are:

1. Approved and monitored, with pre-load vacuum autoclave cycles
2. Operated only by trained personnel, to ensure correct operation, testing, monitoring and documentation

Steam sterilisation of solids

Steam sterilisation relies on steam contact with all the surfaces that need to be sterilised, so that the latent heat of steam is released when the steam condenses on the item.

Air inside tubing and glass vessels is not easily displaced by steam and can prevent steam from contacting all surfaces. Modern autoclaves displace air inside by creating a vacuum so that steam under pressure can penetrate all parts of the load.

Preparation

- Only use special autoclave bags that will not melt. (Heat and pressure within an autoclave will melt conventional plastics and damage the autoclave).
- Place items to be autoclaved in a loosely sealed autoclave bag or a vessel that will easily allow air to be withdrawn and steam to contact all the surfaces of the items.
- It is important to place agar within a suitably sized steel basin or specially designed stainless steel container. (Heat and pressure will melt agar, which can easily spill and damage the autoclave).



Purpose-designed autoclave bag

Steam sterilisation of liquids

While cheap and easy, steam sterilisation of liquids in glass containers is a very inefficient process. The steam must condense on the surface of the vessel, and the energy must therefore be transmitted through the glass in order to bring the liquid up to boiling point and be held there for a minimum of 10 minutes.

Consequently, volumes over one litre may never reach boiling point, so we recommend that users provide media in volumes less than one litre.

Autoclaves are fitted with a temperature probe that monitors the temperature of the load. The temperature probe enables the autoclave to sense when the holding times for liquids may begin. It is imperative that the liquid container into which the temperature probe is placed is the same volume and temperature as the largest container in the load to be sterilised.

Preparation

- Present liquids to be autoclaved in volumes less than 500mls. If necessary, split larger volumes into multiples of less than 500mls. Alternatively, consider other methods of liquid sterilisation, such as filter sterilisation.
- Ensure the volume of COLD water in the container with the temperature probe is the same as the volume of the largest container of media (i.e. the largest volume will be the rate limiting step).
- Take care when placing the temperature probe in the container of cold water. (Temperature probes are very delicate and easily broken.)

USE IT How to use a Class II biological safety cabinet (BSC)

There is an excellent Australian video on the safe use of Class II biological safety cabinets (BSCs), which you can access on the Canvas Containment Course web page.

Before you start work, ask yourself

- Is this cabinet certified?
- Is it functioning correctly?
(Lights are on, airflow is operating etc.)
- Is it clean and clear of unnecessary items?
- Do I have the right equipment to perform my task?(Make a checklist)
- Is the seat at the right height for me?
(Your back should be straight and your forearms at 90 degrees to the front vertical face of the cabinet)



Class II biological safety cabinet

STORE IT/RECORD IT

Storage in the laboratory

All imported or transferred risk goods and GMOs developed onsite must be stored in labelled cabinets, refrigerators or freezers. The purpose of these labels is to warn other users of the presence of restricted goods.

How to work within the BSC

- Switch on the cabinet air blower at least 3-5 minutes before starting work.
- Move your arms into/out of the BSC slowly so as not to disrupt the containment air curtain.
- Ensure other people are not coming and going around the cabinet, or opening and closing doors to the room, as these movements can also disrupt the air curtain.
- Keep your arms and hands still for about one minute after placing them inside the BSC. Don't rest your arms across the front grille or the room air may flow into the sterile work area.
- All movements inside the cabinet should be at least 10cm from the front grille.
- Do not use Bunsen burners in a Class II cabinet as the heat from the flames creates turbulence and will disturb air movements in the hood.
- Do not overload the working surface with equipment as the equipment will reduce clear aseptic workflows and will also impede airflow within the cabinet.
- Operate as much as possible in the centre of the work surface.
- Operate aerosol-generating equipment in the rear of the cabinet.
- Discard used pipettes into a horizontal, disinfectant containing tray kept within the cabinet.
- Disinfect the work surfaces and the interior walls before and after use.
- Don't take potentially contaminated material out of the cabinet until you have decontaminated the surface with an approved disinfectant.
- Do not store equipment inside the BSC.



If stored in a fridge or freezer, the risk goods must be stored in a marked secondary container such as a larger plastic box. The purpose of the labelled secondary container is to ensure all risk goods are in a single place and that laboratory personnel return risk goods to a single nominated container.

Keeping laboratory records

Laboratories are required to keep a laboratory register of imported or transferred risk goods within **SciTrack**. Items are to be recorded as whole containers.

If a kit is composed of multiple constituents, the number of containers may be recorded in the free-text field. SQERM records are to include:

1. An accurate description of the item
2. The date of importation or transfer
3. **BACC** number for restricted imports
4. **Central Register number**
5. SciTrack barcode number
6. HSNO approval number (if applicable)
7. The exact storage location of the item
8. Information about date of consumption and/or destruction

Laboratories are not required to maintain files with copies of transfer approvals but may request copies of transfer approvals and BACCs from the **authorised signatory in charge of import/transfer**. All users should be able to add items to the SciTrack shared folder, but note that modification or removal of items is restricted to authorised personnel.

Documentation of transfer within containment facilities

If the material is transferred to another lab in the containment facility, the receiving lab must keep a record of:

- The storage location in your laboratory
- Record of any disposal
- MPI approval for any transfer to another containment facility or re-export



MOVE IT

Importing restricted biologicals into New Zealand

If you need to import restricted biological material into the country for your research project, there is a process that must be followed to meet the requirements of the University and the Ministry for Primary Industries (MPI).

Restricted biologicals include New Organisms, Unwanted Organisms, genetically modified organisms (GMOs), biological materials that present a potential biosecurity risk, serums, albumins and cell lines.

The process for importing New Organisms, Unwanted Organisms and GMOs is more complicated than the process for importing other restricted biologicals. See the step-by-step instructions for researchers in Appendix 1.

Transferring/exporting restricted biologicals

If you need to transfer restricted biologicals into University of Auckland containment facilities (from within New Zealand), or transfer them out to other containment facilities within New Zealand and overseas, you must obtain prior MPI approval. Applications for MPI approval are obtained via the authorised signatories in each containment facility. The process is outlined in Appendix 2.

Full information on importing and transferring restricted biologicals is available from the Health, Safety and Wellbeing Service.

Packaging restricted biologicals for transfer/export

All restricted material transferred between University facilities, shipped to other facilities in New Zealand or exported to overseas destinations must be packaged according to regulatory requirements for road or air transport. In some cases use of a DG (dangerous goods) courier is mandatory. Please ask your TTL/Floor Manager for

details. Specialist packaging is available at some stores or through a DG courier.

Unpacking imported restricted biologicals

Whenever a restricted biological product, micro-organism or cell culture is imported into a containment or transitional unit, the person who unpacks the goods – normally the designated laboratory person (DLP) – must check that:

- The integrity of the package has been maintained
- The package has been delivered to the right place and person
- All appropriate documentation has been included and is in order
- Primary and secondary packaging is intact and there is no leakage
- Numbers and descriptions correlate with accompanying documentation
- The identity of the items is correct and there are no additional items
- The packaging material is disposed of appropriately. If it is unpacked on a clean bench, it can be recycled.

You must forward all paperwork to the **authorised signatory in charge of import/transfer** immediately shipment is received. This will enable the facility to apply for any retrospective permits if the usual notifications fail.

If the package is leaking, incorrect items have been sent, or the documentation is missing/incorrect, notify the authorised signatory immediately.

Disposal of restricted biologicals

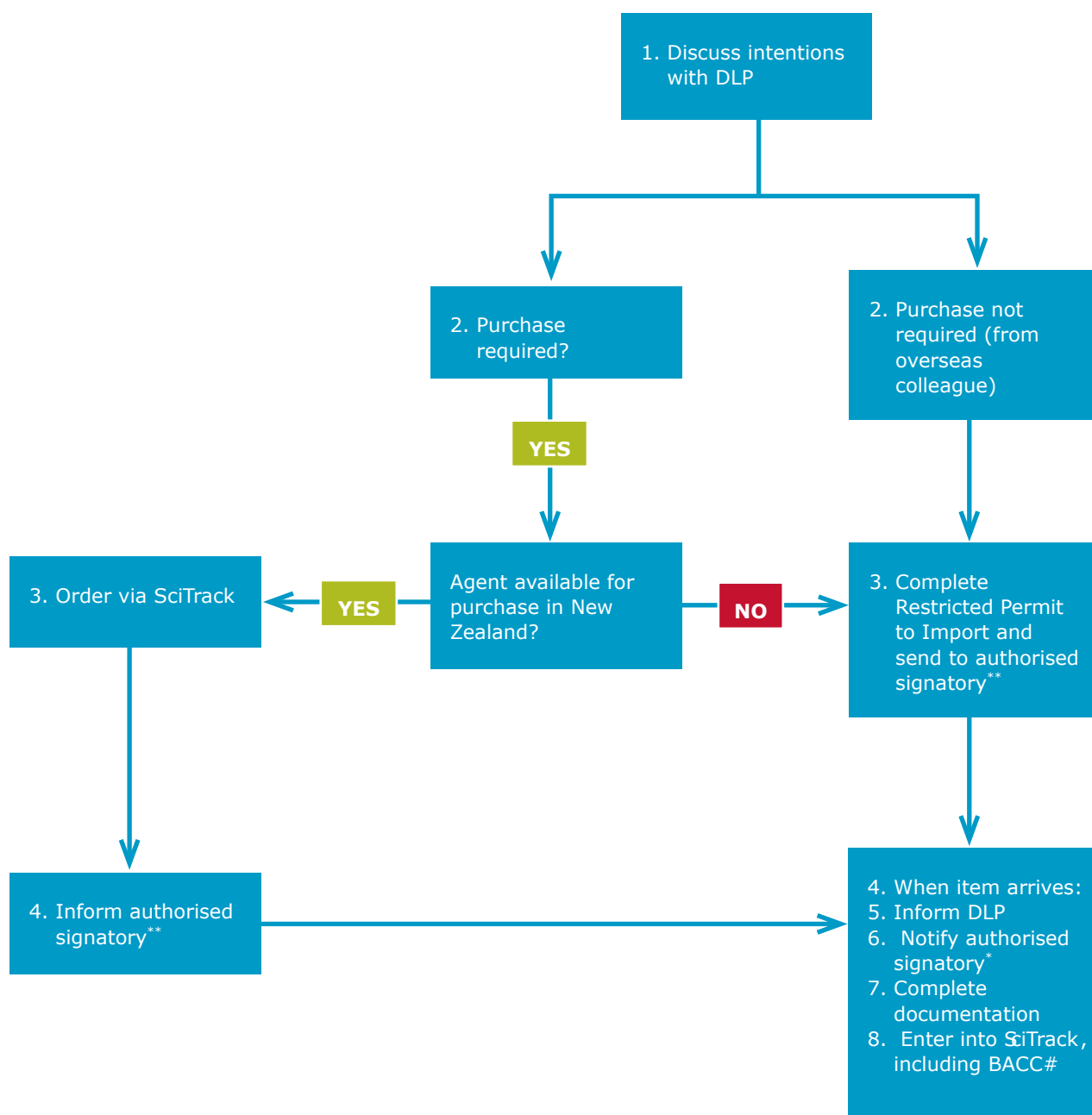
Please ensure that all restricted biologicals are correctly disposed of when you have completed your work. After decontamination (refer to "Chemical Decontamination" on page 2 or "Autoclaving" on page 4), the item needs to be disposed of in an approved biohazard bag.

APPENDIX 1 Import of GMOs, New Organisms and Unwanted Organisms



* Researchers in the Faculty of Medical and Health Sciences (FHMS) should send requests and completed permits to containment@auckland.ac.nz

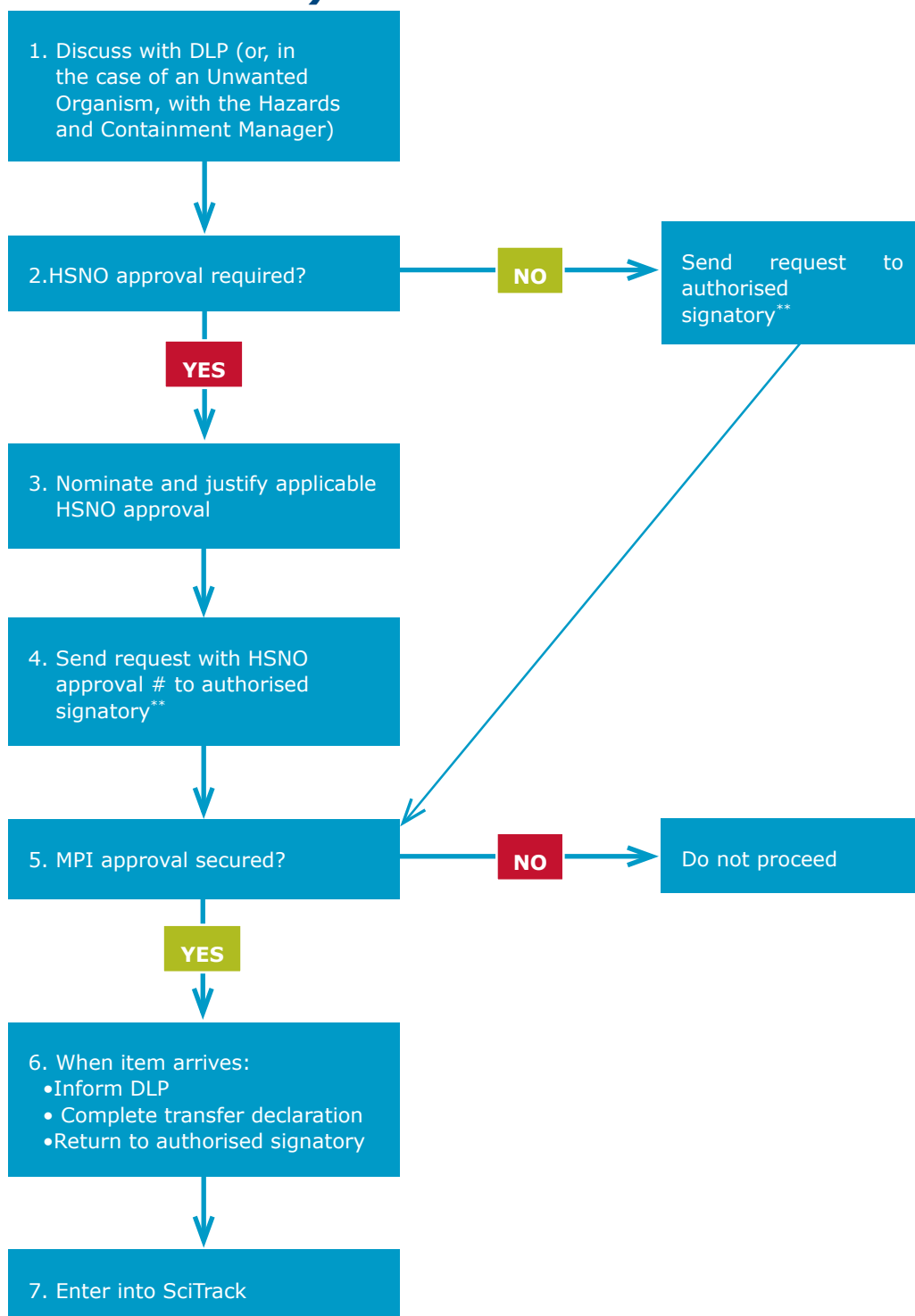
Import of Restricted Biologicals*



* Restricted biologicals (other than Unwanted Organisms, New Organisms and GMOs) include serums, albumins and cell lines. For full information on the import process, please refer to "Importing Restricted Biologicals".

** Researchers in the Faculty of Medical and Health Sciences (FHMS) should send requests and completed permits to containment@auckland.ac.nz

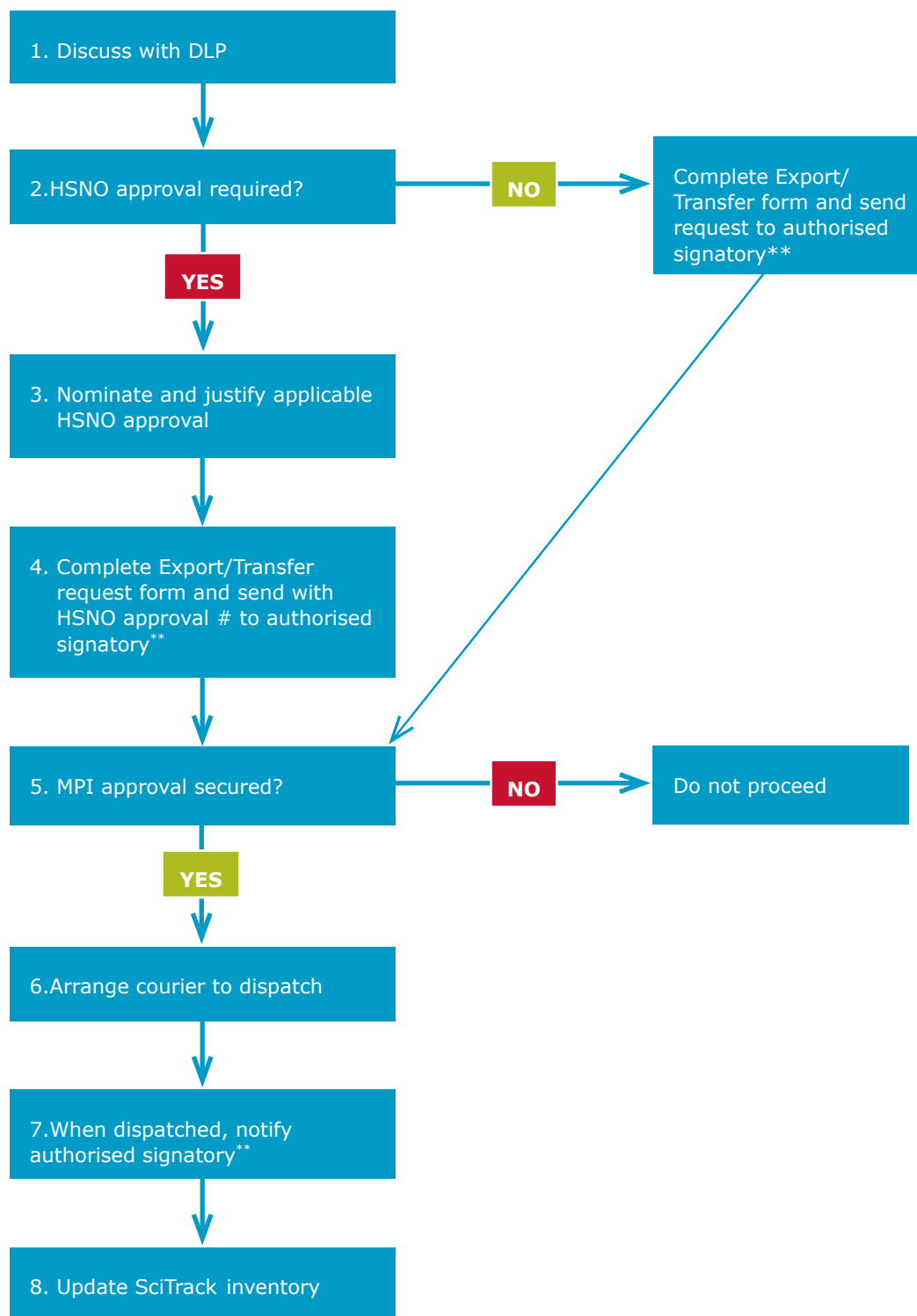
APPENDIX 2 Transfer in of Restricted Biologicals* (within New Zealand)



* Restricted biologicals include Unwanted Organisms, New Organisms, GMOs, serums, albumins and cell lines. For full information on the transfer process within New Zealand, please refer to "Transferring/Exporting Restricted Biologicals"

** Researchers in the Faculty of Medical and Health Sciences (FHMS) should send requests and completed permits to containment@auckland.ac.nz

Transfer Out of Restricted Biologicals*



* Restricted biologicals include Unwanted Organisms, New Organisms, GMOs, serums, albumins and cell lines. For full information on the transfer/export process, please refer to "Transferring/Exporting Restricted Biologicals".

** Researchers in the Faculty of Medical and Health Sciences (FHMS) should send requests and completed permits to containment@auckland.ac.nz

APPENDIX 3: BACC form

Biosecurity Authority/ Clearance Certificate

Pursuant to Sections 25 and 26 of the
Biosecurity Act 1993

Ministry for Primary Industries
Manatū Ahu Matua



C2017/40283

← **BACC number**

CUSMOD Release No.:

BACC No.: B2017/33942

NZCS Entry No.: 86151379

All Biosecurity Requirements Met?

NO

Each Authority contained in this document identifies the goods that are covered by the Authority, a Transitional Facility that you are authorised to take the goods to, and any conditions which the authorisation is subject to.

Removal of these goods to a place other than the Transitional Facility authorised, or otherwise than in accordance with the conditions specified, is an offence.

Any clearance or Authority that is contained in this document, and that relates to agricultural compounds or veterinary medicines, also constitutes permission to remove these goods under the conditions contained within the Agricultural Compounds and Veterinary Medicines Act 1997.

Authority Issued To: UNIVERSITY OF AUCKLAND, SCHOOL OF BIOLOGICAL SCIENCES THOMAS BUILDING,
3A, AUCKLAND, Auckland

Importer: UNIVERSITY OF AUCKLAND, SCHOOL OF BIOLOGICAL SCIENCES THOMAS BUILDING,
3A, AUCKLAND, Auckland

Agent: Federal Express Pacific Inc, Laurence Stevens Drive, Auckland Airport, Auckland

Contact: Melisa Lolohea

Arrival Method: Flight: NZ108 **Date:** 20/01/2017

IDENTIFIERS:

B/L:778190548256 Sub B/L:778190548256:

← **TRACKING NUMBER**

AUTHORITY

To be taken to: The University of Auckland - SBS, 3-5 Symonds St, Auckland, Auckland

For: Further action at Transitional Facility as per IHS/Permit/CTO Direction

By: Transitional Facility, -

Authority Conditions:
2016060646

Authorising Inspector: Hodgson, Colette

Location: Target Evaluation (Cargo)

Date: 01/02/2017

GOODS COVERED BY THIS AUTHORITY:

No.	Line Type	Country of Origin	Line Details
1	Biologicals	USA	Other biological products, Biological, 1.000 unit(s), 1.000 package

Line Identifiers: B/L:778190548256; Sub B/L:778190548256:

Issued By: XXXXXX

Location: Target Evaluation (Cargo)

Signed: _____

Signing Date: 01/02/2017

GLOSSARY

Approved chemical disinfectants are those disinfectants (listed on page 2) with proven efficacy as ratified by the United States Environmental Protection Authority (EPA), peer reviewed journals or by European testing regimes.

Authorised signatory in charge (of imports/transfers) means a designated person in any of the following roles: floor manager, facility manager, laboratory manager, technical team leader, technical manager or technical officer.

BACC means Biosecurity Authority Clearance Certificate (also known as the cargo number).

Central register means an electronic register of all transfers and restricted imports, showing details of item and vendor as well as BACC number and MPI approval number, enabling this information to be cross-referenced with documentation stored on SciTrack.

Designated laboratory person (DLP) means the trained person in each research group who has been given the authority to receive purchase requests made in SciTrack and to make a formal request for a purchase order via Shared Transaction Centre. In containment and transitional facilities DLPs will have additional training to enable them to scrutinise documentation for restricted items and provide support to researchers.

A **genetically modified organism (GMO)** is an organism modified by in vitro manipulation for which approval to import or to develop is required under the Hazardous Substances and New Organism (HSNO) Act. GMOs must be held in a Ministry of Primary Industries-approved containment facility as a primary control condition of HSNO approval. Movement of GMOs from a containment facility requires prior specific approval under the HSNO Act.

Hazard approver (HA) means the trained persons in each faculty who have been given the authority to approve the purchase of restricted and controlled items in PeopleSoft.

High hazard infectious micro-organisms means micro-organisms in Risk Group 2 that do have a route of infection via the respiratory tract. The University of Auckland Biological Safety

Committee will give special consideration to spills outside biosafety cabinets before approving work with such microorganisms, so that spill clean-up processes can be proactively identified.

High organic load liquid wastes means wastes such as bacterial and eukaryotic cell cultures (where $>10^5$ eukaryotic cells per ml), and blood/body fluids that have a high organic load, making chemical sterilisation difficult.

Low hazard infectious micro-organisms means microorganisms of Risk Group 1 and 2 that do not have an accepted route of infection via the respiratory tract.

Low organic load liquid wastes means wastes such as low density cell cultures (as a guideline $<10^5$ cells per ml), trap waste, used media and culture supernatants amenable to chemical treatment.

Principal investigator: The holder of an independent grant administered by the University and the lead researcher for the grant project, usually in the sciences, such as a laboratory study or a clinical trial. The phrase is also often used as a synonym for "head of the laboratory" or "research group leader." The Principal Investigator is responsible for assuring compliance with applicable University standards and procedures, and for the oversight of the research study and the informed consent process. Although the PI may delegate tasks, they retain responsibility for the conduct of the study.

Restricted Permit to Import means those Permits to Import whose post-entry conditions require the imported material to be held in a New Zealand Ministry of Primary Industries approved transitional or containment facility.

Restricted biologicals include New Organisms, Unwanted Organisms, genetically modified organisms (GMOs), and risk biologicals.

Risk biologicals are biological materials that present a potential biosecurity risk and include serums, albumins and modified cell lines. Unmodified human cell lines are not considered risk biologicals by MPI.

SciTrack means the University's electronic register of all GMOs and restricted imports.



Approved by: Associate Director, Health, Safety and Wellbeing

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