FIEBRE Standard Operating Procedure F.15c			
Title	**Selection, preparation, and shipping of bacterial and fungal isolates to the microbiology reference laboratory**		
SOP Reference		***Version***	***Date of effect***
F.15c		1.1.1	[pending]

SOP Development

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Review Tracker

Due date for next review	**Reviewer name**	**Signature**	**Date reviewed**

Revision History

Version No.	**Effective date**	**Reason for change**

SOP User Confirmation

I acknowledge that I have read, understood and agree to follow this SOP

#	Name (print)	Signature	Date
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1. **Title: Selection, preparation, and shipping bacterial and fungal isolates to the microbiology reference laboratory**

2. **Purpose:** To describe the procedures for selecting, preparing, and shipping bacterial and fungal isolates from FIEBRE study sites to microbiology reference laboratory in New Zealand.

3. **Responsible staff:** FIEBRE laboratory and co-ordination staff [site-specific]

4. **Background & Rationale:** A blood culture will be performed for each patient enrolled in the FIEBRE study, and urine culture will be performed for a subset of patients. For positive cultures, laboratory staff at the study site will identify the microorganism/s present, and will test for antimicrobial susceptibility. Then staff will ship a sample of each isolate to the reference laboratory in New Zealand, where MALDI-TOF will be used for confirmation and quality control.

   This SOP explains how to select which isolates to ship, and the preparation and shipping requirements for transfer from each site directly to the reference laboratory.

5. Supplies and Materials

- Sample logbook (paper and ODK)
- A list of cultures and boiled lysates to send; this will be provided by LSHTM
- 2 mL cryovials containing the bacterial isolates, clearly labelled with a barcoded specimen ID and the species name
- 0.5 mL cryotubes containing microorganism DNA, clearly labelled with a barcoded specimen ID and "DNA"
- Cryoboxes
- Gloves (single-use latex or vinyl)
- Cryogloves for handling dry ice
- Biohazard disposal system
- Lab surface disinfectant (e.g. Biocleanse)
- Box map
- Packaging, shipping labels, and dry ice (to be provided by courier)

6. Procedures:

6.1 Storage of samples on site (see also FIEBRE SOP F-08a)

6.1.1 Microorganisms isolated from blood and urine cultures (both true positives and those thought to be contaminants) should all be stored in 2 mL cryovials in a -80°C freezer.

6.1.2 Boiled lysates should be stored in 0.5 mL O-ring cryovials in a -80°C freezer.

6.2 Selecting samples for shipment

6.2.1 The determination of which samples to ship will be made in discussion with study coordinators and the LSHTM team.

6.2.2 New Zealand has strict rules about which organisms it can import. Every microorganism imported requires an import permit.

6.2.3 Some organisms are not permitted to be shipped to New Zealand; these are considered "unwanted organisms". Examples of organisms so far identified and their

status are given in Appendix A. ***The list in Appendix A is only an example, and is not comprehensive; before shipping, please check your site's organism list with FIEBRE coordinators as in item 6.2.7.***

6.2.4 FIEBRE site staff may not be able to identify all isolates to species level. A bacterial/fungal isolate that is only identified to genus level is not covered by the import permit.

6.2.5 Where bacteria/fungi have not been fully identified but are not likely to be dangerous (HG3/4) or not likely to be identified as "unwanted organisms", study staff should give them a presumptive name. This will be done in discussion with study coordinators and LSHTM FIEBRE staff.

6.2.6 Unwanted organisms cannot be shipped to New Zealand. In cases where an unwanted organism has been identified, or if the isolates have only been identified to genus/group **and** they have the potential to be HG3, HG4 or "unwanted organisms" they must be shipped as boiled lysate for identification by DNA analysis (see SOP F15e).

6.2.7 When preparing shipments, please discuss with the LSHTM laboratory coordinator (currently Victoria Gould [Victoria.Gould@lshtm.ac.uk]), your site's coordinator and PI if relevant, and the FIEBRE scientific program coordinator (Heidi Hopkins) to determine which isolates and which boiled lysates to include in the shipment.

6.2.8 Only ship microorganisms that have import permits.

6.3 Classification for shipping

The transportation of infectious substances is divided into the following categories:

6.3.1 **Category A**: an infectious substance which is carried in a form that, when exposure to it occurs, is capable of causing permanent disability, life threatening or fatal disease in otherwise healthy humans or animals. This definition is supplemented by an indicative list of pathogens, which include HIV and hepatitis B viruses (but not hepatitis C virus), when in the form of cultures but does not encompass specimens from patients suspected of having these infections.

6.3.2 **Category B:** any infectious substance that does not meet the criteria for inclusion in category A. These are assigned to UN 3373. This would include specimens from patients with known or suspected HIV, HBV or HCV infections.

6.3.3 Most specimens, including all boiled lysates will be shipped as Category B. Some isolates, such as *Salmonella* Typhi*,* must be shipped at Category A. Category A and category B shipments have different packaging and documentation requirements. You will need to discuss with the LSHTM laboratory coordinator and site coordinators to determine which isolates will need to be shipped under which category.

Category B specimens

6.4 Packaging and labelling of Category B samples for shipment

6.4.1 **Packaging of dry ice samples –** all Category B shipments must be triple packaged in accordance with International Air Transport Association (IATA) guidelines.

6.4.1.1 The primary receptacle is the 0.5 mL or 2 mL leak-proof cryotube holding each sample. These should be securely sealed. Ship the cryotubes in the plastic cryoboxes where they are currently stored. As an extra precaution, place each cryobox inside a Ziploc bag; pack each bag with a suitable absorbent material, such as blue roll, to absorb any spills that may occur during transport (Figure 1 A and B).

6.4.1.2 The secondary container is a leak-proof 650- bio-pouch bag, which will be supplied by the courier. Pack a maximum of 4 cryoboxes (within their individual Ziploc bags) into each bio-pouch (figure 1 C).

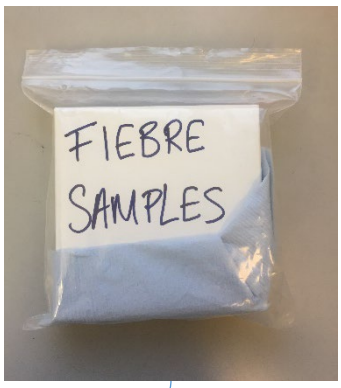
6.4.1.3 The third layer is a rigid outer box, which will hold the dry ice container. The courier will provide these boxes and the dry ice. The boxes should come marked with the appropriate hazard labels (see figure 2).

Figure 1: Example of how cryoboxes should be packed for shipping.

A

Place cryoboxes in a Ziploc bag with enough blue roll to absorb all the contents in case of a spill

B



Cryobox and absorbent material within a Ziploc bag

C

Place a maximum of 4 cryoboxes in 1 Bio-pouch (the “secondary container”). The bio-pouches will be provided by the courier.

6.5 Labelling for Category B outer boxes (see figure 2)

6.5.1 Sender's name and address (your own contact information)

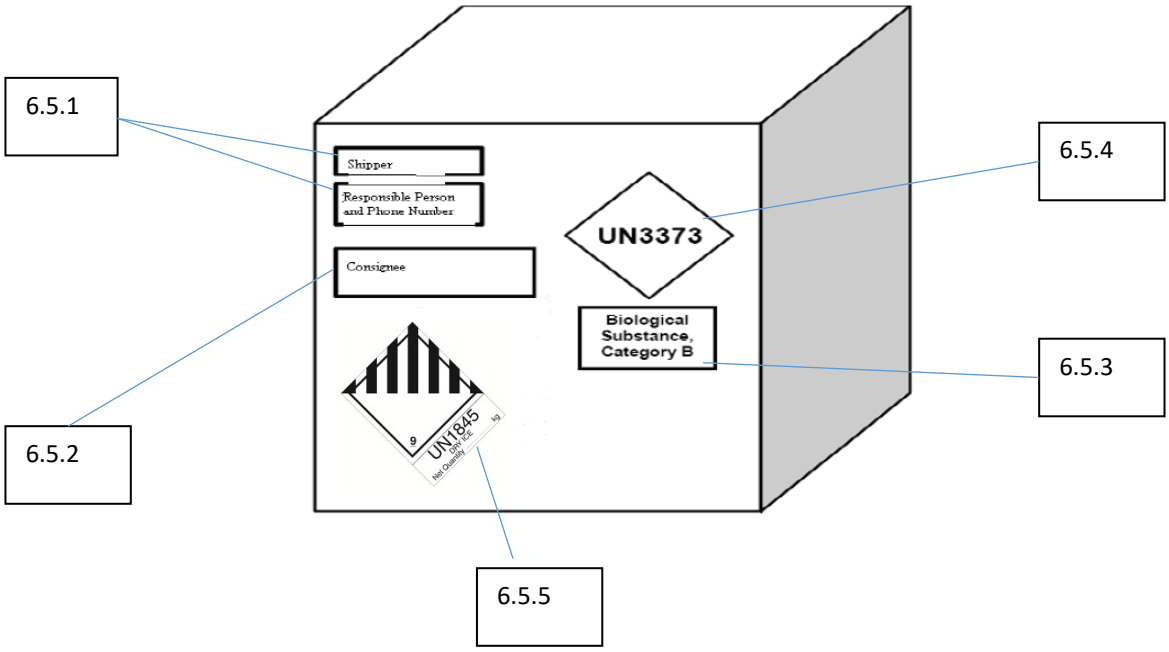
6.5.2 Recipient's name, address and contact number (see 6.1)

6.5.3 The words “Biological Substance, Category B”

6.5.4 UN3373 label (supplied by the courier)

6.5.5 A class 9 label, along with the words “UN1845 Dry ice” and the weight of dry ice in kilograms

Figure 2. Labelling of Category B shipment



6.6 Documentation required for Category B shipment

6.6.1 Place two copies of each of the following documents in a transparent envelope and affix to the outer box:

a. A customs invoice written on site-specific headed paper. Include the number and volume of each sample type shipped. State clearly that these specimens

have no commercial value. Contact the LSHTM lab coordinator for a template if needed.

b. A certificate of donation on site-specific headed paper. Contact the LSHTM lab coordinator for a template if needed.

c. Copies of all the relevant import permits.

Place a third copy of each document within the outer package.

Category A specimens

6.7 Packaging for Category A packaging

6.7.1 **Packaging of dry ice samples –**Packaging for Category A infectious substances **must** meet UN performance requirements for Class 6.2 substances as shown by design type testing. These are known as **UN type approved packaging** and they are certified and marked accordingly. Packaging used for Category A infectious substances must be specifically approved for Class 6.2 goods (either primary receptacle or secondary packaging). This can be ensured by purchasing from a specialist company such as AirSea containers where suitable products are listed under Category A packaging and are appropriately certified and marked (see 6.7.4)

6.7.2 All Category A shipments must be triple packaged in accordance with International Air Transport Association (IATA) guidelines.

6.7.3 The primary receptacle is the 2 mL leak-proof cryotube holding each sample. These should be securely sealed with waterproof tape.

6.7.4 The secondary container must be watertight and leak-proof e.g. Biotubes and Biojar available in various sizes from AirSea containers (www.airseadg.com) appropriate to volumes being shipped. Several cushioned primary receptacles may be placed in one secondary packaging, but sufficient additional absorbent material must be used to absorb all fluid in case of breakage. Place the sealed primary receptacles within any supplied pouch, or wrap them in absorbent material, and then place them within the secondary container.

6.7.5 For air transport the limits per package are as follows: • 50ml or 50g for passenger aircraft • 4L or 4kg for cargo aircraft. Any primary receptacle with a capacity of more than 50 ml must be oriented in the outer packaging so that the closures are upwards. Orientation labels (“UP” arrows) must be affixed to two opposite sides of the outer packaging.

6.7.6 The third layer is a rigid outer box, which will be the dry ice container. The courier will provide these boxes and the dry ice. The boxes should come marked with the appropriate hazard labels (see Figure 3).

Place the secondary packaging within the outer packaging along with suitable cushioning material. The outer packaging should bear the United Nations packaging specification marking, which indicates that the packaging has passed the performance tests to the satisfaction of the competent authority.

6.8 Labelling for Category A outer boxes (see Figure 3)

6.8.1 Sender's name, address and emergency contact number (your own contact information)

6.8.2 Recipient's name, address and emergency contact number (see 6.1)

6.8.3 The words “UN 2814 INFECTIOUS SUBSTANCE, AFFECTING HUMANS”

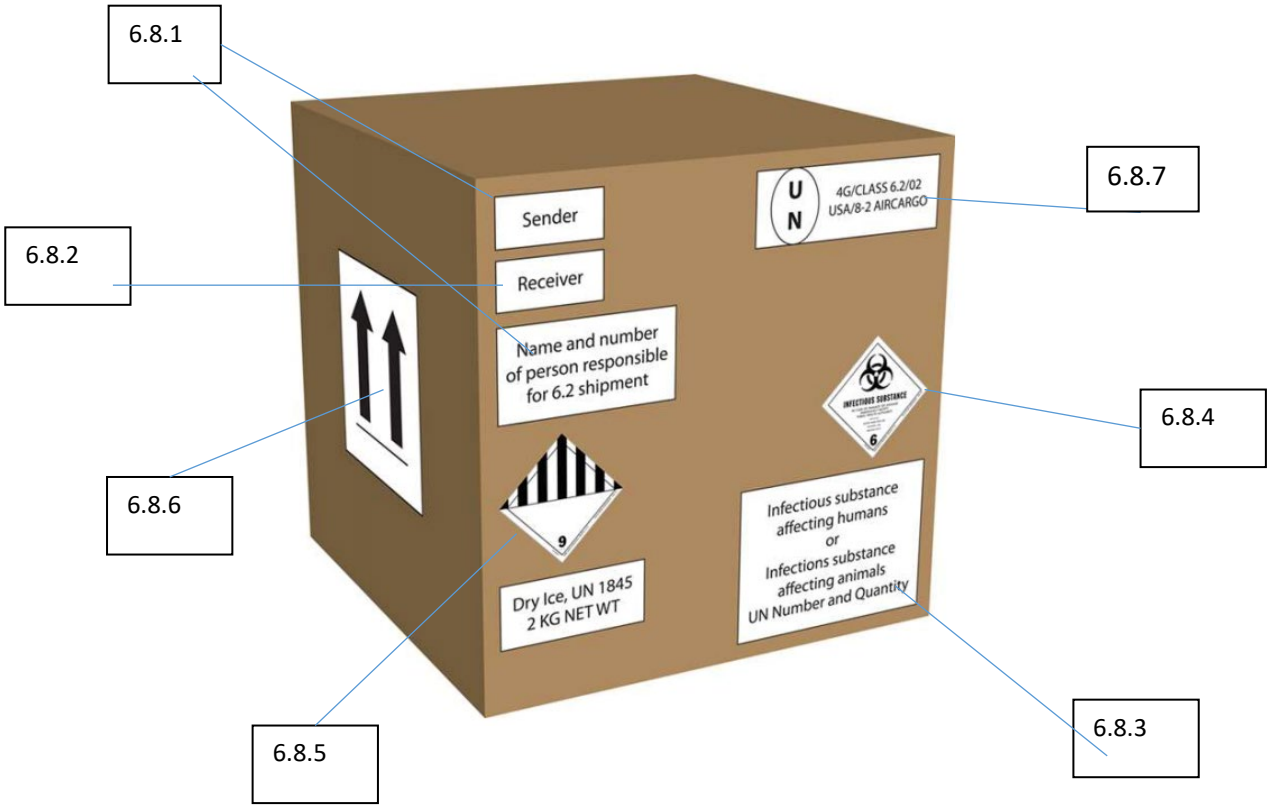
6.8.4 Hazard warning label for Class 6.2 - Infectious substances

6.8.5 A class 9 label, along with the words “UN1845 Dry ice” and the weight of dry ice in kilograms

6.8.6 Packages containing liquids must display "Package Orientation" labels.

6.8.7 UN packaging specification mark

Figure 2. Labelling of Category A shipment



6.9 Documentation required for Category A shipment

6.9.1 Place two copies of each of the following documents in a transparent envelope and affix to the outer box.

a. A packing list – an itemised list of contents, including the number of tubes and the sample volume in each tube.

b. A customs invoice written on site-specific headed paper. Include the number and volume of each sample type shipped. State clearly that these specimens have no commercial value. Contact the LSHTM lab coordinator for a template if needed.

c. A certificate of donation on site-specific headed paper. Contact the LSHTM lab coordinator for a template if needed.

d. Copies of all the relevant import permits.

e. For transport by air, complete an Air Waybill and a Shipper's Declaration for Dangerous Goods. Contact the LSHTM lab coordinator for a template if needed.

6.10 Recipient address for agreed **isolates**:

Attention of: James Ussher

Microbiology Building
University of Otago
720 Cumberland Street
Dunedin 9054
New Zealand

Phone: 0277078576

7. Appendix A

The list of microorganisms shown is only an example, and is not comprehensive; before shipping, please check your site's organism list with FIEBRE coordinators as in item 6.2.7.

Table 1: Bacteria/fungi identified to group/genus only from both blood and urine cultures. A presumptive name has been suggested for all non-HG3 bacteria/fungi

Bacteria/fungi identified	Presumptive name	Notes	NZ import	UK Hazard Group
a-haemolytic streptococcus other than pneumo	*Streptococcus oralis*		Listed on page six of Import Permit 2020076604	2
Acinetobacter spp	*Acinetobacter baumanii*		Listed on page one of Import Permit 2020076604	1
Alcaligenes spp	*Alcaligenes faecalis*		Listed on page one of Import Permit 2020075585	2
Diphtheroids	*Corynebacteria striatum*		Listed on page one of Import Permit 2020075585	2
Enterococcus spp.	*Enterococcus faecalis*		Listed on page three of Import Permit 2020076604	2
Klebsiella spp.	*Klebsiella pneumoniae*		Listed on page three of Import Permit 2020076604	2
Coagulase Negative Staph	*Staphylococcus epidermidis*		Listed on page five of Import Permit 2020076604	1
Group A Streptococcus	*Streptococcus pyogenes*		Listed on Import Permit 2020076604	
Group B Streptococcus	*Streptococcus agalactiae*		Listed on Import Permit 2020076604	
Group D Streptococcus	*Streptococcus bovis*		Listed on Import Permit 2020076604	

Micrococcus spp.	*Micrococcus luteus*		The *Micrococcus luteus* will have to come in on the restricted permit as there is no EPA determination.	1
Viridans group Streptococcus	*Streptococcus oralis*		Listed on Import Permit 2020076604	1
Staphylococcus spp.	*Staphylococcus epidermidis*		Listed on Import Permit 2020076604	
Non-typhoidal Salmonella	*Salmonella enterica*		Listed on Import Permit 2020076604	
Salmonella spp.	*Salmonella enterica*		Listed on Import Permit 2020076604	
Yeast		Need to give a presumptive name	Please check any fungal isolate with the project co-ordinator before sending.	
gram negative cocci		possible HG3	Send boiled lysates- "Non-viable unfixed cell lysates listed on page two of Import Permit 2019073661".	
Bacillus (gram +ve)		possible HG3	Send boiled lysates- "Non-viable unfixed cell lysates listed on page two of Import Permit 2019073661".	
Non-lactose fermenting		possible "unwanted organism"	Send boiled lysates- "Non-viable unfixed cell lysates listed on page two of Import Permit 2019073661".	
Unknown		Possible HG3	Send boiled lysates- "Non-viable unfixed cell lysates listed on page two of Import Permit 2019073661".	

Table 2: Bacteria/fungi identified to species level from blood and urine cultures

	Notes	NZ import	UK Hazard group
Acinetobacter baumanii		Listed on Import Permit 2020076604	1
Burkholderia pseudomallei	UK Schedule 5 – unwanted in NZ	Send boiled lysates- "Non-viable unfixed cell lysates listed on page two of Import Permit 2019073661".	3
Burkholderia cepacia		Listed on Import Permit 2020076604	
Candida lusitaniae		Listed on Import Permit 2020076604	2
Citrobacter freundii		Listed on Import Permit 2020076604	1
Cryptococcus neoformans		Listed on Import Permit 2020076604	2
Enterobacter aerogenes		Listed on Import Permit 2020076604	2
Enterobacter cloacae		Listed on Import Permit 2020076604	2
Escherichia coli		Listed on Import Permit 2020076604	2
Haemophilus influenzae		Listed on Import Permit 2020076604	2
Hafnia alvei		Listed on Import Permit 2020076604	2
Klebsiella pneumoniae		Listed on Import Permit 2020076604	2
Methylobacterium mesophilicum		Listed on Import Permit 2020076604	1
Ochronobacter antropi		Listed on Import Permit 2020076604	1
Propionibacterium acnes		Listed on Import Permit 2020076604	1
Proteus mirabilis		Listed onImport Permit 2020075585	2

Pseudomonas luteola		Listed on Import Permit 2020075585	1
Pseudomonas aeruginosa		Listed on Import Permit 2020076604	
*Salmonella enterica (*Typhi*)*	UK Schedule 5	*S. enterica for import docs (Salmonella enterica* Listed on Import Permit 2020076604	3
Salmonella paratyphi		*S. enterica for import docs (Salmonella enterica* Listed on Import Permit 2020076604	
Salmonella Typhimurium	Certain phage types only	Listed on Import Permit 2020076604	2
Staphylococcus epidermis		Listed on Import Permit 2020076604	1
Staphylococcus aureus		Listed on Import Permit 2020076604	2
Streptococcus mitis		Listed on Import Permit 2020076604	
Streptococcus oralis		Listed on Import Permit 2020076604	2
Streptococcus pneumoniae		Listed on Import Permit 2020076604	2
Streptococcus pyogenes		Listed on Import Permit 2020076604	2
Talaromyces marneffei		Please check all fungal isolates with project co-ordinator before sending	2

Appendix B

WHO guidance on shipping. Including indicative list of what needs to be shipped as Category A.

WHO_CDS_EPR_2007_2cc.pdf

Appendix C

NZ import permits**. Current as of Feb 2021. Please check expiry dates before shipping**

Import_Permit_2020 076604.pdf

Import_Permit_2020 075585.pdf

Import_Permit_2020 076881.pdf

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