

# Containment of *Mycobacterium marium* within a containment level 2 aquatics area

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## Introduction

With the use of fish in research continuing to rise, the requirements to adapt facilities for the housing of multiple different species has grown.

In many instances the introduction of new aquatics species requires room alterations, building management system amendments, compliance with regulations differing to that of the mammalian species and structural modifications.

The University of Cambridge created 2 Containment Level 2 (CL2) aquatics facilities (Figures 1 and 2), housing multiple species of fish infected with *Mycobacterium marinum*. Here we define the PPE used to prevent the spread of *Mycobacterium marinum* in a multi roomed facility, the CL2 system design solutions, training, fish feeding and screening of fish within the facility.



Figure 1. CL2 Room 1.

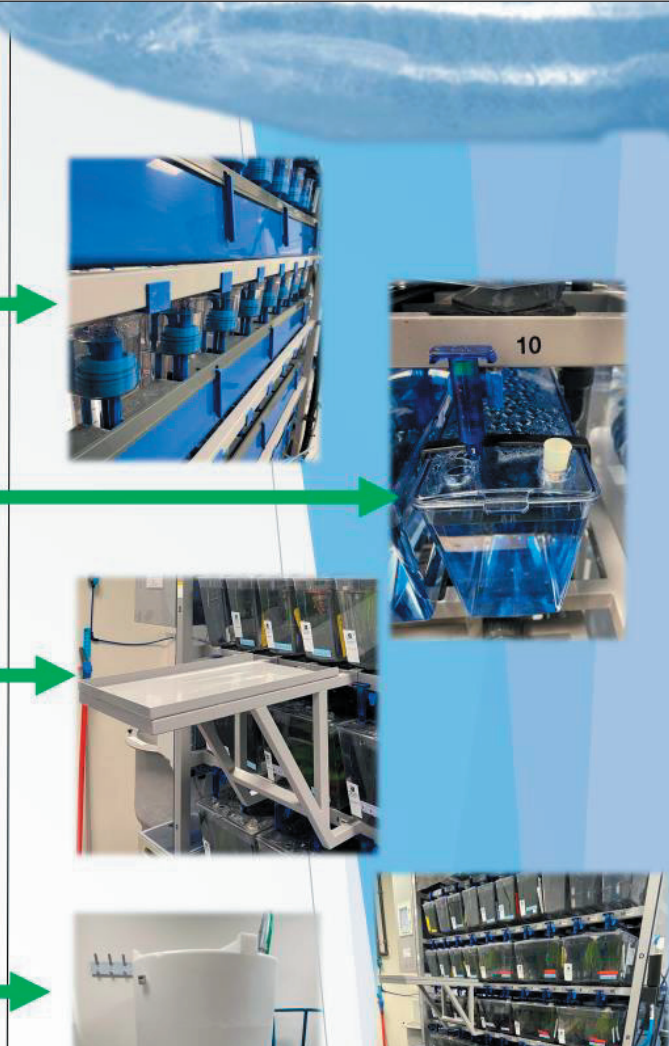






Figure 2. CL2 Room 2.

## Personal Protective Equipment

Potential routes of contamination are ingestion, inhalation and inoculation with particular risk of infection through contact of skin, eyes, and mucous membranes. To ensure the safety of the staff within the CL2 area and prevent contamination to our other fish rooms, the following PPE has been implemented in line with Health and Safety Executive (HSE) recommendations, this is donned within a clean zone of CL2 and removed before leaving CL2:

- Conducting procedures not requiring the removal of a tank from the rack – CL2 specific shoes, 2 pairs of gloves, disposable lab coat, face shield.
- Conducting procedures requiring removal of a tank from the rack – CL2 specific shoes, 2 pairs of gloves, Tyvek with boots due to the increased risk of splashing contaminated water, face shield.

Differences Between a CL2 System and a Standard Housing System	Solutions Used Within University of Cambridge CL2 Facility		
Aerosols and splashing must be avoided to prevent cross contamination and staff exposure to pathogens	<ul style="list-style-type: none"> <li>• Rubber bungs fit within the tank lids</li> <li>• Standard system modifications to include covers to rack sumps and drains</li> <li>• Clips added to tank lids to prevent the lid from falling off during transport</li> <li>• Mobile shelves are placed on the rack to allow for the removal of the tank without the need to carry the tank to a bench area</li> </ul>		
Waste water must be decontaminated before entering the drainage system	<ul style="list-style-type: none"> <li>• Water from the system and sink is collected in large storage tanks where it is chemically treated for a set period of time before entering the drains</li> </ul>		

## Training facility technicians and users

Within our facilities, staff movement is permitted between rooms and CL2 without cross contamination. This has been achieved by:

- All staff must be trained and assessed with in depth knowledge of the CL2 control measures, health and safety risks and legislation.
- Where possible perform tasks at the end of the day.
- Standard operating procedures and risk assessments must be read and understood by each member of staff before working in the area.
- Code of Practice provided detailing; work conducted in the area, hazards, disinfection processes, control of contamination, process to handle spills, waste disposal, approved disinfectants and key contacts.

## Disinfection and cleaning procedures

- **Water disinfection:** collection within storage tanks and provided chemical treatment before entering drains.
- **Tank cleaning:** cleaning of non infected tanks first. All tanks cleaned using tissue and Reverse Osmosis water (RO) before being autoclaved at 134°C using a validated, efficacy tested autoclave.
- **Non autoclavable tank components:** baffles are cleaned with 70% ethanol for 2 minutes before rinsing with RO water.
- **Nets:** almost all are autoclavable, those which are not are cleaned with 70% ethanol.

## **Fish feeding**

### **Risk:**

Cross contamination of bacteria from infected tank to clean tank.

### **Solution:**

- separate feeding equipment (one for non infected and one for infected)
- feed uninfected fish first, then infected
- unused food should be discarded after each group is fed
- cleaning of feeding equipment after each group is fed
- colour coded feeding equipment and tanks for ease of differentiation between control and infected animals and equipment

## **Screening of fish and swabs for *Mycobacterium marinum***

Screening for pathogens potentially harmful to fish health is routinely performed in an aquatic facility. Within a CL2 facility, the screening performed is not to confirm what pathogens you have, but rather to confirm the processes you have in place are reliably preventing cross contamination.

## **Procedure at University of Cambridge**

- screen control fish every 3 to 6 months
- screening of fish by PCR and histology
- screening of swabs of tank biofilm pre and post filtration
- swabs of room surfaces to confirm room hygiene standards are maintained

## **Further reading**

Zebrafish Journal – *Design and Husbandry Considerations for a Containment Level 2 Aquatic Facility* – Nicola Goodwin and Lynda Westall.