BIOSAFETY STANDARD OPERATING PROCEDURES

COLD-OCEAN DEEP-SEA RESEARCH FACILITY

DEPARTMENT OF OCEAN SCIENCES, MEMORIAL UNIVERSITY

October 22, 2021

Biosafety Orientation

Cold-Ocean Deep-Sea Research Facility (CDRF)

Department of Ocean Sciences

Memorial University of NL

VERSION 3.1

Introduction

The Cold-ocean and Deep-sea Research Facility (CDRF) is a facility of the Department of Ocean Sciences at Memorial University of Newfoundland. In addition to analytical and deep-sea labs, the facility includes an aquatic containment zone for the study of pathogens and invasive species. A variety of viral, bacterial and parasitic organisms, and the host species they affect, will be studied in the containment zone. Additionally, species which are considered invasive to Newfoundland and Labrador will be housed here.

Staff working within the containment zone must be familiar with the biosafety procedures outlined in this manual and the standard operating procedures particular to their own work. Overall guidelines and certification for aquatic containment facilities are managed by the Canadian Food Inspection Agency (CFIA). Materials for this manual are drawn from their *Containment Standards for Facilities Handling Aquatic Animals*, as well as from the biosafety manuals of the Public Health Agency of Canada (PHAC) in collaboration with the CFIA, the World Health Agency (WHO) and other labs. These manuals may serve as a reference to add to the material found herein and should be reviewed by those who will be regularly working with infectious materials.

Containment Standards for Facilities Handling Aquatic Animals
http://inspection.gc.ca/animals/aquatic-animals/imports/pathogens/eng/1312436244596/1322885037191

Canadian Biosafety Standards and Guidelines
http://canadianbiosafetystandards.collaboration.gc.ca/

WHO Biosafety Manual
http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

The "CDRF Biosafety Manual" refers to this orientation material and a collection of biosafety and animal care standard operating procedures (SOPs) that will be assigned to you based on your individual training needs and job requirements. This material compliments the biosafety program of Memorial University. For comprehensive information on MUN's biosafety program, please refer to the following link:

http://www.mun.ca/health_safety/biosafety/

This includes MUN biosafety SOPs which give greater detail on the requirements for working with biohazardous material at Memorial University.

Research and Program Intent

The containment facility of the CDRF has many unique uses. Aquatic Containment level 3 (AQC3) labs are rare in Canada even more so with a local source of cold ocean water. Research projects include:

- Research on invasive marine species (tunicates, mollusks, fish and crustaceans).
- Controlled *in vivo* and *in vitro* pathogen challenge experiments of host-pathogen interactions.
- Vaccine and therapeutant development.
- Isolation and propagation of endemic pathogens (viral, bacterial, fungal, animal and microsporidian).

- Identification of disease resistant fish strains and development and testing of molecular diagnostics for pathogen exposure, carrier / disease status.

Disease challenges will generally be conducted with intra-peritoneal or intra-muscular injections of cultured virus or bacteria. Fish will be maintained in 500 L tanks during the experiments and removed within the disease challenge necropsy rooms for surgery or disection.

To prevent accidental infection, fish will be sedated before any invasive procedures including injection and the tanks covered. Preserved tissues will pass out of the disease challenge rooms into the dry labs for further processing. Tissues will not leave the containment area unless fixed, or otherwise processed to inactivate infectious organisms. Infectious material may be transported to another containment lab following the safe transport guidelines detailed in SOP CDRF.B.3010 provided the receiving lab is certified to work with the material.

Cell and tissue culture will occur within the dry lab spaces. Manipulations of infectious material will occur in BSCs utilizing procedures to minimize the production of aerosols as described in the biosafety manual. All personnel working in the containment zone will undergo training in biosafety practices as outlined in the manual.

List of pathogens

Most aquatic pathogens are not zoonotic and will not cause disease in healthy humans, however containment operations are still required to prevent release to the environment, other labs and farms.

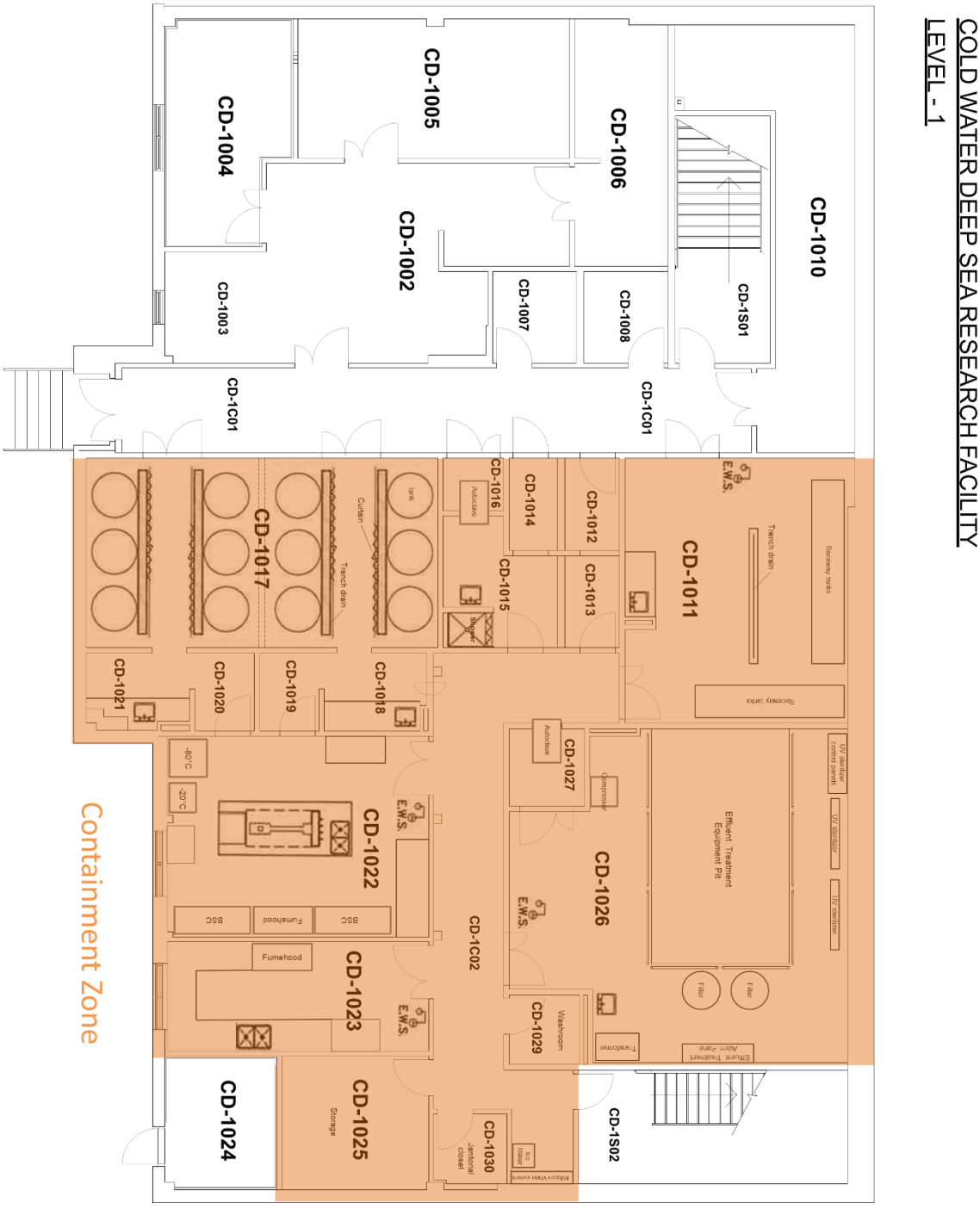
- Primary (short term or continuing plans to use)
    - *Lepeophtheirus salmonis*
    - Typical and atypical *Aeromonas salmonicida*
    - *Piscirickettsia salmonis*
    - *Moretiela viscosa*
    - *Yersinia ruckeri*
    - *Renibacterium salmoninarum*
    - *Vibrio* spp.
- Secondary (long term plans to use)
    - Infectious salmon anemia virus (ISAV)
    - Nodavirus
    - *Loma morhua*
- The CFIA to be notified through a program change request before any new pathogen is introduced to the facility or there is a significant change in procedures.

Animal species manipulated in the facility

- Atlantic salmon (*Salmo salar*)
- Atlantic cod (*Gadus morhua*)
- Rainbow trout (*Oncorhynchus mykiss*)
- Lumpfish (*Cyclopterus lumpus*)
- Zebrafish (*Danio rerio*)
- Sablefish (*Anoplopoma fimbria*)

Building layout

The CDRF is a two-story building with the containment zone, deep-sea labs and water treatment on the ground floor. The upper floor contains additional laboratory space (including microscopy, histology and cell sorting equipment), as well as office space. The area marked in orange in the figure below indicates the containment zone (CZ) and room numbers of the first floor. Room CD-2016 is a CL-2 lab, although this manual applies chiefly to activities in the Aquatic Containment CZ.



Containment features

The containment zone is designed to prevent unauthorized access and the release of pathogens or invasive species to the community and the environment. It follows the construction guidelines for AQC3 labs itemized in the CFIA's *Containment Standards for Aquatic Facilities.* Key features include:

- Secure access and operational separation from other areas of the CDRF and the OSC.
- Clean lockers for street clothing and dirty lockers for lab clothing within anterooms.
- Surfaces of floors, walls, ceilings and bench-tops resistant to moisture, easy to decontaminate and non-absorbent.
- A waste water treatment plant to treat all water used within the containment labs.
- Inward directional airflow and double door entrance and exit interlocks.
- Two pass-through autoclaves for sterilization of biohazard waste.
- Dedicated disease challenge and necropsy rooms.
- Foot baths and hands-free washing sinks.
- Two biosafety cabinets.

By following the instructions laid out in this manual you will:

- *Protect yourself from becoming ill with laboratory acquired infections.*
- *Prevent the release of disease organisms or invasive species from the CDRF to the environment or community.*
- *Protect your experiments, and the experiments of others from becoming contaminated.*

Glossary

- BSC: Biological safety cabinet
- BSO: Biosafety officer
- CFIA: Canadian Food Inspection Agency
- CL: Containment level
- CZ: Containment zone
- LAI: Laboratory acquired infection
- PHAC: Public Health Agency of Canada
- PI: Principal investigator
- PPE: Personal protective equipment
- RA: Research assistant
- SOPs: Standard operating procedures
- WWTP: Wastewater treatment plant

Roles and responsibilities

Any personnel entering the containment area must be aware of the biosafety requirements particular to their role. Training is detailed below. Until new personnel are fully trained, they must be supervised by trained staff. All persons entering the facility must follow the regulations and procedures described in this manual, the associated SOPs and their training.

Any user determined to be disregarding these procedures will be reported to the facility manager and their supervisor. They will first receive a warning and subsequently may require retraining or lose CDRF access privileges.

The following describes the responsibilities for personnel working at the CDRF. See the contact information for the current information on CDRF staff.

Facility manager

The facility manager oversees biological safety at the facility. This includes maintaining records and oversight on building and equipment maintenance, biosafety inspections and training. The facility manager is responsible for keeping the biosafety manual and SOPs up to date and submitting annual recertification documentation to the CFIA.

Institutional Biosafety Officer (BSO)

At the university, there is also an institutional biosafety officer who sets and enforces biosafety policy for the university. The BSO inspects labs to ensure compliance with university policy and all biosafety-related legislation, sets training requirements, issues university biosafety lab approvals and reviews submissions to regulatory agencies such as the CFIA. The BSO can be contacted at bso@mun.ca. They are the primary contact for biosafety training and the importation, storage, permitting and transfer of pathogens

CDRF containment research associate

The containment RA, as well as other CDRF staff regularly working in the containment zone, must be trained in biosafety techniques and containment features of the facility. They are responsible for supervising and training any visitors to the facility, including visiting researchers and students. The containment technician must maintain all equipment and pathogen inventories and provide these records to the facility manager. They are responsible for insuring all materials leaving the facility are properly decontaminated.

Visiting scientists, students and staff

Visiting staff must first be granted permission to access the containment zone by the facility manager. This includes PIs, students, janitorial, and maintenance personnel. Once approved, they must be trained by CDRF staff (generally the containment RA) on approved biosafety techniques and the SOPs they will be using. In addition to biosafety training, they should be trained in general laboratory safety and chemical safety for the SOPs they are using. Until training is complete, visitors must always be supervised by trained personnel. After training is complete, they may enter the facility as needed. PIs must obtain biosafety certificates before beginning work in the CZ and share the approved certificate with the CDRF manager. They are responsible for ensuring that staff and students under their supervision are trained in all project specific biosafety protocols and that this training is recorded.

Short-term visitors

Guests and infrequent visitors, such as contract workers are allowed in the facility under the supervision of trained personnel with the approval of the facility manager. Visits should be kept to a minimum and group sizes kept small. Visitors are limited to only those areas in the facility that are necessary and must follow the SOPs for entry and exit as well as PPE.

Training

Anyone wishing to work within the containment zone must have completed the Memorial biosafety training course and have a valid worker registration form.

http://www.mun.ca/health_safety/OHSMS/BSMS/BiosafetyTraining.php

Specialized training relevant to the risks entailed in their work will also be set by the facility manager. This additional training is largely divided into SOPs for people working in the wet lab and / or the dry labs. Basic and specialized training SOPs are indicated on the *CDRF Training* form, which is to be completed and submitted to the facility manager.

1. A training checklist will be provided indicating the training SOPs required.
2. Each SOP must be demonstrated by a trained operator and initially performed by the new user under supervision.
3. When training is complete and has been signed-off by the facility manager, an individual may work unsupervised in the containment area. Training will be documented, and training records maintained by the facility manager.
4. Note, you may only work within approved areas, which may exclude the disease challenge rooms.
5. Retraining for emergency procedures should occur annually. The SOPs should be reviewed by experienced operators and demonstrated again to personnel who have not performed the SOP within the year. The online biosafety training course must be redone every five years.

The “Biosafety Manual” consists of this orientation plus a collection of SOPs specific to biosafety as well as some SOPs from the animal care collection with biosafety specific material. *Containment Standards for Facilities Handling Aquatic Animal Pathogens* from the CFIA must be read.

Containment Standards for Facilities Handling Aquatic Animals
http://inspection.gc.ca/animals/aquatic-animals/imports/pathogens/eng/1312436244596/1322885037191

Biosafety training is also available online from PHAC. Although not particular to aquatic labs, these courses are an excellent accompaniment to the biosafety manual.

PHAC Biosafety e-learning
http://www.phac-aspc.gc.ca/lab-bio/res/blk-acb/index-eng.php

Conditions of Access

Obtaining a biosafety certificate

Research granting agencies require that operations involving cell cultures, bacteria, mycoplasma, fungi, viruses, and parasites be monitored by an institutional Biosafety Committee. Before beginning this work, obtain a biosafety certificate by contacting the biosafety officer at bso@mun.ca. Further information can be found at

http://www.mun.ca/health_safety/biosafety/

The biosafety certificate must list the room in the CDRF where work with the biohazard organism will take place. The rooms are:

CD1022: Main containment zone dry lab. Includes -80°C and liquid nitrogen storage.

CD1017 A & B: Disease challenge rooms.

CD1011: Invasive species room.

CD1022: Small dry lab.

All personnel working in the CDRF with these organisms should be listed on the certificate.

A copy of the approved biosafety certificate should be provided to the facility manager. Failure to complete and renew biosafety certificates will result in a disallow to conduct work in the CZ and the possible denial of funds for the project by RGCS.

Access permission and security

Initial access must be obtained from the facility manager directly or through the containment RA. If access is required to the disease challenge rooms, this must be specified. Short term access for site visits, equipment or facility repair, cleaning etc. is allowed so long as the visitor is accompanied by trained staff, however those wishing to conduct research in the facility should be listed on an active biosafety certificate.

Access is through an electronic swipe card, which will be activated once approval is granted. Visitors and untrained personnel must be accompanied by trained staff. Detailed instructions on entry and exit procedures can be found in protocol *CDRF.B.1010 Entry and exit procedures for personnel*.

1. All doors entering the containment zone are signed with the international biohazard warning sign. This indicates the current containment level and emergency contact numbers. Signage will be kept up to date.
2. Personnel entry occurs only through CD-1012 and exit through CD-1016. These doors are interlocked with CD-1013 and CD-1015 respectively. All other doors are to remain locked.
3. Entry of large items is allowed through rooms CD-1011, CD-1017A and B if needed by following protocol *CDRF.B.1020 Entry and exit procedures for large items.*
4. Access to the disease challenge rooms must be specifically authorized.
5. Children are not allowed within the laboratory working areas.
6. Animals, other than those involved in the work of the laboratory, are not allowed.

Working alone

Because the containment zone is a restricted access zone, those working alone in the facility outside of normal working hours should check in and out with the facilities service technician or other on-site staff when using the facility. During working hours, the containment RA will periodically inspect the lab when it is in use. A sign-in and sign-out board will be mounted at the containment perimeter, and all personnel authorized to use the facility will be required to indicate their presence in the facility.

Medical

As part of training, a list of pathogens being used in the lab, as well as their associated human symptoms of infection, if any, will be provided. This information is also available at the entrance to the containment zone. This includes pathogens that the trainee is not planning to work on. Although the risk of zoonotic infection from aquatic organisms is generally less than terrestrial experimental animals, this will be highly dependent on the pathogens present at any given time. Familiarize yourself with the symptoms associated with all the pathogens in use in the facility.

A risk assessment of new pathogens entering the laboratories will determine if immunization is required. At this time, no special immunizations or surveillance are required. If you have any medical conditions or disabilities that may limit your ability to work in the biocontainment zone, you must inform the facility manager and should discuss this with your family doctor. This would include conditions that make donning PPE (e.g. glove allergies) problematic. Alternative procedures will be discussed in these cases. Persons with immune dysfunction, pregnancy or other conditions may acquire laboratory infections more easily or suffer more severely from a LAI. Exclusion from the CZ or limiting work to low risk activities may be necessary. This will depend upon a review of the pathogens and procedure in use and a risk assessment conducted by the PI and the facility manager.

When working in the CZ:

1. Cover all wounds, scratches and cuts with waterproof dressings.
2. Do not work with biohazard material or infected animals when lack of sleep, fatigue or illness will prevent you from being alert and coordinated.
3. In the event of an accidental exposure, follow the incident and spill procedures outlined in the biosafety manual. Inform the facility manager who will assist in reporting to the local OSH representative (listed in contacts).

Common practices

The following practices are considered essential habits and must be followed. They are detailed further throughout the laboratory and biosafety SOPs.

- Eating, chewing gum, drinking, smoking, storing food, storing personal belongings, applying cosmetics, and inserting or removing contact lenses are prohibited within the CZ.
- Oral pipetting of any substance is prohibited in any laboratory.
- Use of needles, syringes and other sharp objects should be strictly limited. Needles should not be bent, sheared, recapped or removed from the syringe; they should be promptly placed in a puncture-resistant sharps container.
- Work surfaces must be cleaned and decontaminated with a suitable disinfectant at the end of the day and after any spill of potentially biohazardous material.

- Hands must be washed frequently (after handling infectious materials, after removing gloves, and before leaving the laboratory).
- Open-toed and high-heeled shoes must not be worn in the laboratory.
- Long hair is to be tied back so that it does not contact hands, specimens, containers or equipment.
- Gloves must be worn when handling infectious materials.
- A BSC must be used for procedures with potential for producing infectious aerosols with high concentrations or large volumes of infectious materials.

Next Steps

1. PIs should fill out and submit a biosafety certificate application.
    a. This can be obtained at http://www.mun.ca/health_safety/biosafety/.
    b. Provide a copy of this certificate to the CDRF facility manager.
2. All workers should register as a biohazard worker with the BSO and do the online training
    a. Worker registration Appendix 6: http://www.mun.ca/health_safety/OHSMS/BSMS/Biosafetyprogramforms.php.
    b. Online course: http://www.mun.ca/health_safety/OHSMS/BSMS/BiosafetyTraining.php.
3. Begin your CDRF training by reading the required biosafety SOPs and arranging a time with the containment RA for onsite training. You will be provided a *CDRF Training* form to complete.

The staff at the CDRF are always available to help you with whatever assistance you may need to make your research safe and successful.

Version history

Version	Date	Authors	Notes
1.0	2014-03-19	Stephen Hill	First version, based on biosafety manuals. CFIA first submission.
2.0	2014-07-23	Stephen Hill	Revisions based on CFIA review. Addition of program intent material, requirement to read CFIA manual.
2.1	2015-03-12	Stephen Hill	Revisions based on BSO review. Clarifying institutional BSO vs. facility manager / internal BSO.
2.2	2015-05-05	Stephen Hill	Minor edits.
2.3	2016-03-01	Stephen Hill	Added in requirement for institutional biosafety training course and worker registration. **CFIA approved 2017.**
3.0	2018-04-11	Stephen Hill	Review before recertification. Minor edits and more references to MUN biosafety program.
3.1	2019-03-27	Stephen Hill	Minor changes. Clarifications of roles.

Memorial University of Newfoundland
Department of Ocean Sciences
Cold-Ocean Deep-Sea Research Facility

Containment zone entry and exit procedures for personnel

Version	Date of last revision
3.4	2019-03-11

Description

Entry to the containment zone (CZ) is limited to authorized personnel and normally occurs through only one entry point (CD-1012 / CD-1013) and one exit point (CD-1015 / CD-1014). These controls have the primary aim of preventing the release of infectious material or invasive species outside of the lab. The secondary goal is to prevent pathogens from being carried into the containment zone where they may contaminate ongoing experiments. These procedures apply to everyone entering the containment zone, including visitors and janitorial or maintenance staff and are required training for any person working in the containment zone. The following instructions are basic instructions for entry into the lab areas. Specialized instructions, for large items and animals are found in the following SOPs.

For entry and exit of large items or animals, refer to SOPs *CDRF.B.1020* and *CDRF.A.7010.*

For transport of pathogens refer to SOP *CDRF.B.3010.*

Glossary and terms

- **BSO: Biosafety officer**
- **CZ: Containment zone**
- **PPE: Personal protective equipment**
- **SOP: Standard operating procedure**
- **“Clean”: This refers to clothing, spaces and items that have not and should not make contact with laboratory biologicals. E.g. street shoes and clothing.**
- **“Dirty”: Clothing, spaces and items that may have come into contact with laboratory biologicals. E.g. PPE, almost all of the interior space of the CZ.**

Safety and responsibilities

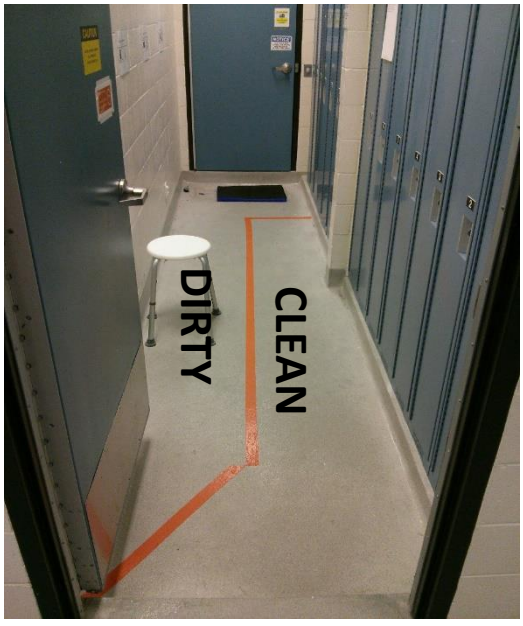
- **All personnel must first obtain permission to access the CZ from the facility manager or the containment RA as detailed in the biosafety manual introductory material (conditions of access, roles and responsibilities).**
- **Training in SOP *CDRF.B.2010* on personal protective equipment.**
- **Anyone who has not been given access and been trained must be accompanied at all times by trained personnel.**

Protocols

Within the CZ, one change of lab clothing is required to enter the dry lab spaces. Secondary PPE or a separate change is required when working in the wet lab spaces. This process will add time to your work routine; plan accordingly and do not skip steps.

Entry to containment zone dry lab spaces

1. **Entry to the CDRF CZ clean change room is through room CD-1012.**
   While in the CZ, never leave doors propped open.
2. **Indicate that you are IN on the sign-in board and fill out the log-book with name, date, time of entry and the reading on the pressure gauge.**
   This is absolutely required for fire safety. If you are accompanying visitors indicate + the number of visitors." The log book will be kept for 3 years.
3. **Use your key pass card to unlock the door. If the positive pressure alarm is on, do not enter.**
   This door is interlocked with all the other antechamber doors such that no two doors may be open at the same time. Do not enter the CZ through any other doors. Notify CDRF staff if the alarm is on.
4. **Open the door and use the visual aid to confirm that there is inward air movement.**
   Tissue or streamers attached to the upper door frame should be drawn inwards towards the CZ.
5. **Within the corridor, areas are marked with orange tape where you may only make contact with laboratory footwear. Do not step in these 'dirty' areas with clean footwear.**



Entry

Exit

6. **Remove outerwear, jewelry and personal effects and store in a CLEAN pass-through locker in the clean area.**
7. **Remove street footwear and don lab footwear.**

Protocols

1. **Sign-in by filling out the logbook.**
   This is located on the table opposite the entry door. Record air pressure from the monitor beside the door.
2. **Slide a marker on the in / out board to indicate your presence.**
   This is important for fire safety.
3. **Place stool in front of a clean locker.**
   These are the four lockers closest to the entry door.
4. **Retrieve laboratory footwear from dirty locker and place in front of the stool on the marked dirty area.**
5. **Remove street footwear and reach over into the clean locker to place them for storage. Do not step into dirty marked area.**
6. **Step into lab footwear in the dirty marked area. Only walk in this area now.**
   For staff regularly working in the CZ, a dedicated keep a pair of footwear in CD-1013. For personnel without dedicated footwear, use the waterproof boot covers provided in CD-1013. Place pant legs inside of boots or boot covers. Further details on PPE are in SOP CDRF.B.2010.
8. **Don a pair of inner lab gloves.**
9. **Don a lab coat from your dirty locker, or other suitable outer lab garment.**
   Anyone performing work in the CZ must wear dedicated lab coats or scrubs. These may be purchased from the CDRF or from the vendors listed in CDRF.B.2010. Visitors who are not performing work may wear a disposable or guest lab coat.
10. **Immerse the soles of footwear in foot bath and pass into the CZ corridor by pressing the PRESS TO EXIT BUTTON.**
    Note: If the interlock on the doors needs to be bypassed in an emergency, emergency pull handles can be found next to the exiting doors. The door may catch, especially if people attempt to open more than one door at a time. If this happens, wait several seconds for the lock to cycle and try again.
11. **Before beginning work, don an outer pair of gloves, fitted over gown cuffs and wear appropriate eye protection.**
    Do not handle contact lenses in the CZ.

Brief passage and inspection

If you are entering the CZ for the purpose of inspection and will not be in any way performing experiments, opening tanks or coming into contact with water, it is only necessary to wear primary PPE (lab coat and gloves), however the footbaths must still be used. The footbaths in the wet labs are deeper than in the dry labs, so appropriate footwear with a deep waterproof sole will be needed.

Work in multiple locations

If you work in both the CDRF CZ and other aquatic spaces, such as JBARB or aquaculture sites, plan your day such that the CDRF work is done last. You should avoid work in other aquatic facilities on the same day after working in the CDRF CZ.

Wet lab entry and exit

Since the wet labs are where *in vivo* experiments will take place, and due to the large volumes of potentially contaminated water, entering and exiting the wet lab requires specialized PPE as detailed in CDRF.B.2010. After entering, ensure door is closed behind you. This includes rooms CD-1017 (A & B), CD-1011 and CD-1026. The entrance of the wet labs is separated into a dry and wet area at the threshold of the foot baths.

If a complete change of PPE is indicated by your protocol, follow these steps:

1. **You will already be wearing dry lab PPE.**
   If you are not opening tanks or performing work in the wet lab (e.g. performing inspection), it is not necessary to change PPE. See note on "brief passage" below.
2. **While standing in the dry space, remove dry lab PPE and place on empty hook.**
   Lab coats are located on hooks inside the wet labs with exterior and wet lab coats located on opposite walls.
3. **Exchange footwear for rubber boots. Sit on the stool on the clean side and place feet into boots which are in the wet side.**
   A pair may be purchased from the CDRF, or you may bring in your own pair.
4. **Don wet-lab lab coat or other suitable outer clothing.**
   This is dependent on the task to be performed. If working in tanks, a lab coat with a waterproof bib is recommended. See SOP "CDRF.B.2010 Personal protective equipment" for more information.
5. **Don an outer pair of gloves.**
6. **Reverse this procedure for exiting. Remove outer wear such that it does not contact your dry lab clothing.**
   Remove wet lab gloves. Remove coat or other covers and hang on hangers. Change out of wet lab footwear.

   - **There is a small specimen pass-through for room CD-1018 which may be used to transfer samples in and out of this necropsy space. The pass-through itself should be decontaminated before and after use.**

Exiting the containment zone

7. **Remove outer gloves at your workstation.**
   Remove gloves by pulling one glove into the other and turning the second glove inside out over the first such that bare skin does not touch the outside of the glove. See page 2 of Appendix A for a diagram of this.
8. **Exit through CD-1015, pausing in footbath to sanitize outer soles of footwear.**

Knock before entering CD-1015 change area.

9. **Decontaminate small items, such as glasses and equipment that will be leaving the lab.**
   Set aside in clean locker. See section below.
10. **Remove PPE, including lab coats and safety glasses and store in the dirty lockers.**
    Lab coats and scrubs that require cleaning should be autoclaved before taking off site for laundering. Boot covers should be placed in the waste biohazard bin for sterilization and disposal.
11. **Remove inner gloves and dispose of them in a biohazard container.**
12. **Wash hands with soap and water.**
13. **Remove lab shoes and put on street shoes.**
    As in entry, use the stool to remove dirty boots in the dirty section, reaching over to place in the locker and then stepping into street shoes in the clean section. Do not touch the outside of lab footwear, remove by holding heel with other foot or handling the inside of the shoe or shoe cover. For this reason, lab footwear should be of a closed, slip-on style.
14. **Disinfect hands with hand sanitizer at end of corridor.**
    To clean hands from lab shoe / boot handling.
15. **Re-don street clothes, and gather personal accessories.**
16. **Exit through CD-1016 by using key card.**
    Confirm that the door closes properly behind you. This door is interlocked with the other antechamber doors such that two doors may not be open at the same time
17. **Sign-out in the log-book and indicate that you are OUT on the sign-in board.**

A shower is available in CD-1015, however for routine purposes showering is currently not necessary. The shower should not be used for non-biosafety reasons. Use the shower if heavy aerosol were generated in a non-contained environment in a way that could contaminate through protective clothing onto exposed skin and hair. A large spill would be one example, but this may also include wastewater treatment failure that resulted in aerosol saturated air or heavy washing with hoses of tanks or rooms. If you detect moisture on clothing or skin and hair, use the shower and decontaminate clothing in the autoclave, using the scrubs provided as temporary clothing.

Operation of the intercom

An intercom is available to communicate from outside the CZ by the entrance (CD-1012) to inside the CZ in the corridor (CD-1C02). The intercom is checked annually to confirm correct operation.

1. **The outside intercom may be used to page the CZ RA or other staff inside the CZ. Press the button once to page.**
   A loud tone will sound that can be heard throughout the CZ. The reverse is not true – the inside intercom cannot be used to page the outside one.
2. **The inside intercom is press to talk, release to listen.**
   I.e. you will not be able to hear the outside person if you hold the intercom button down.

Passage of small items and equipment

Small items, samples in sealed containers, equipment and reagents may enter along with personnel.

1. **Avoid bringing in any items that are not needed for your work.**
2. **Gather materials in advance to avoid multiple trips in and out of the CZ.**
   Tools for maintenance and repair are available inside the CZ.
3. **Any item entering or exiting the CZ must be assumed to be contaminated and treated as such, regardless of use within the CV.**
4. **Outside the dirty entrance, CD-1012 is a small station for decontamination that includes alcohol sprays and wipes which may be used on small items entering the facility. A similar wash station in CD-1015 should be used when exiting such items.**
   Alternatively, the pass-through autoclave may be used for certain items provided the autoclave has been previously cycled. Remove any excess packaging on items coming in.

Excerpt from SOP CDRF.B.2010, list of PPE by activity type

Entry to dry lab	●	○	●		●	○						
Work with infectious materials	●	●	●	●	●							
Entry to wet lab	●	●	●			●						
Wet lab hosing down tanks / room			●	●		●		●	●			
Surgery	●	●	●	●		●	●			●		
Necropsy	●	●	●	●		●	●			○		
High risk of contamination from splashing		●	●	●		●	●	○	○		●	

Recommend PPE for selected activities. ● Mandatory ○ As required

Appendix A:

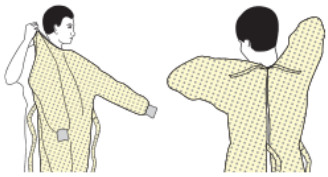
Guide to donning and doffing PPE. PPE required may be different from below.

SEQUENCE FOR PUTTING ON PERSONAL PROTECTIVE EQUIPMENT (PPE)

The type of PPE used will vary based on the level of precautions required, such as standard and contact, droplet or airborne infection isolation precautions. The procedure for putting on and removing PPE should be tailored to the specific type of PPE.

1. GOWN

- Fully cover torso from neck to knees, arms to end of wrists, and wrap around the back
- Fasten in back of neck and waist



2. MASK OR RESPIRATOR

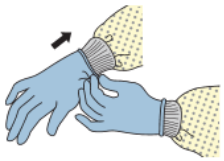
- Secure ties or elastic bands at middle of head and neck
- Fit flexible band to nose bridge
- Fit snug to face and below chin
- Fit-check respirator

3. GOGGLES OR FACE SHIELD

- Place over face and eyes and adjust to fit

4. GLOVES

- Extend to cover wrist of isolation gown

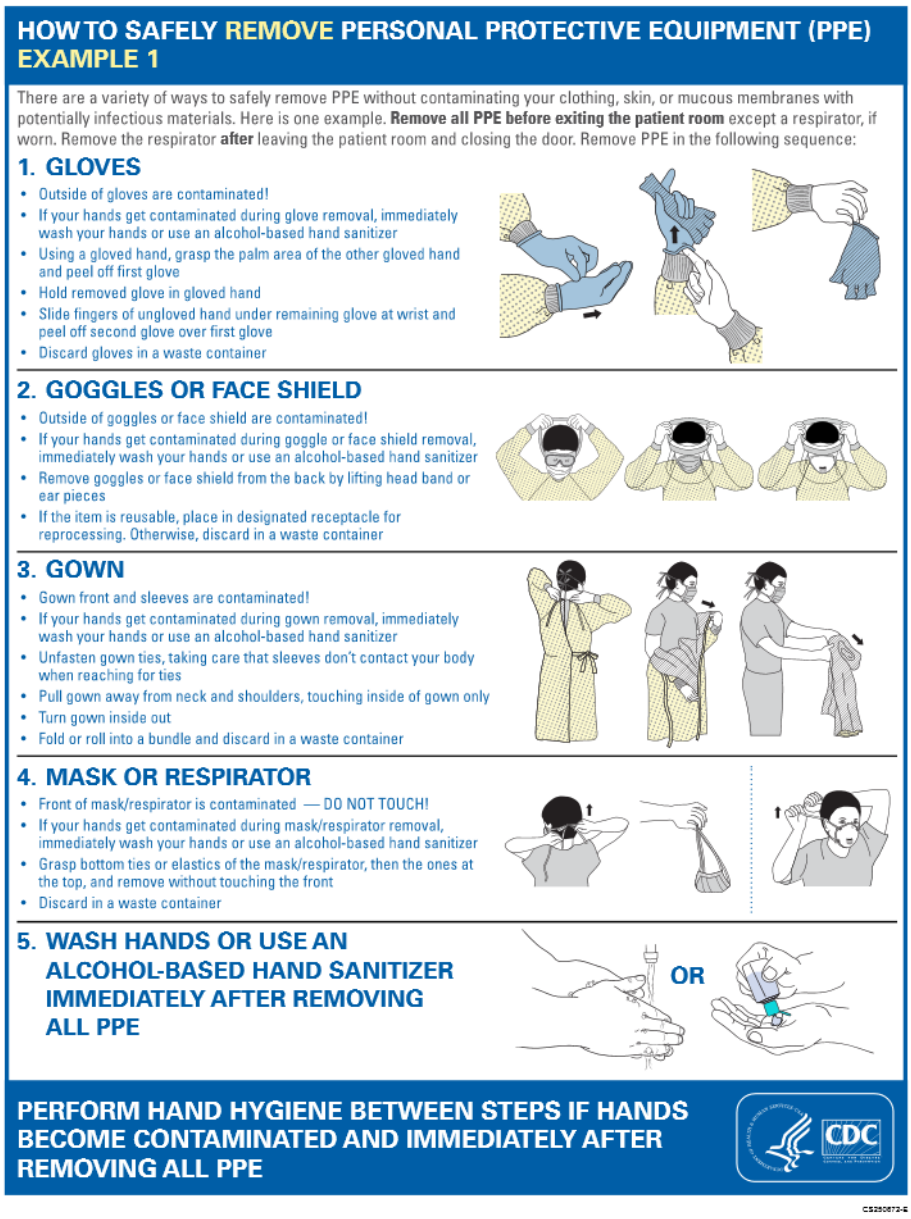


USE SAFE WORK PRACTICES TO PROTECT YOURSELF AND LIMIT THE SPREAD OF CONTAMINATION

- Keep hands away from face
- Limit surfaces touched
- Change gloves when torn or heavily contaminated
- Perform hand hygiene

CDC

CS250672-E



HOW TO SAFELY REMOVE PERSONAL PROTECTIVE EQUIPMENT (PPE)
EXAMPLE 1

There are a variety of ways to safely remove PPE without contaminating your clothing, skin, or mucous membranes with potentially infectious materials. Here is one example. **Remove all PPE before exiting the patient room** except a respirator, if worn. Remove the respirator **after** leaving the patient room and closing the door. Remove PPE in the following sequence:

1. GLOVES
- Outside of gloves are contaminated!
- If your hands get contaminated during glove removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Using a gloved hand, grasp the palm area of the other gloved hand and peel off first glove
- Hold removed glove in gloved hand
- Slide fingers of ungloved hand under remaining glove at wrist and peel off second glove over first glove
- Discard gloves in a waste container

2. GOGGLES OR FACE SHIELD
- Outside of goggles or face shield are contaminated!
- If your hands get contaminated during goggle or face shield removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Remove goggles or face shield from the back by lifting head band or ear pieces
- If the item is reusable, place in designated receptacle for reprocessing. Otherwise, discard in a waste container

3. GOWN
- Gown front and sleeves are contaminated!
- If your hands get contaminated during gown removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Unfasten gown ties, taking care that sleeves don't contact your body when reaching for ties
- Pull gown away from neck and shoulders, touching inside of gown only
- Turn gown inside out
- Fold or roll into a bundle and discard in a waste container

4. MASK OR RESPIRATOR
- Front of mask/respirator is contaminated — DO NOT TOUCH!
- If your hands get contaminated during mask/respirator removal, immediately wash your hands or use an alcohol-based hand sanitizer
- Grasp bottom ties or elastics of the mask/respirator, then the ones at the top, and remove without touching the front
- Discard in a waste container

5. WASH HANDS OR USE AN ALCOHOL-BASED HAND SANITIZER IMMEDIATELY AFTER REMOVING ALL PPE

OR

PERFORM HAND HYGIENE BETWEEN STEPS IF HANDS BECOME CONTAMINATED AND IMMEDIATELY AFTER REMOVING ALL PPE

CDC

References

1. Canadian Biosafety Standards, 2nd edition (2015).
2. *Containment Standards for Facilities Handling Aquatic Animal Pathogens*. (Canadian Food Inspection Agency, Biohazard Containment and Safety Science Branch, 2010).
3. *Laboratory Biosafety Manual*. (World Health Organization, 2004).

Version history and notes

Version	Date	Author	Notes
1.0	2014-01-31	Stephen Hill	Created. Based on a review of CFIA and PHAC biosafety manuals[1–3].
1.1	2014-05-14	Stephen Hill	Edits from KG, MR. CFIA opening submission.
2.1	2014-07-24	Stephen Hill	Corrections from first CFIA review. Returned from scrubs to lab coats. Added info on intercoms.
2.2	2015-03-12	Stephen Hill	Corrections from institutional BSO. Added CDC guide.
2.3	2015-05-11	Stephen Hill	Changes to order of exit, hand wash is now last step.
2.4	2015-10-25	Stephen Hill	Version accepted by CFIA.
3.0	2016-10-25	Stephen Hill	Work in progress – updating to clarify change procedures separating wet and dry labs.
3.1	2016-12-16	Stephen Hill	Clearing comments, minor revisions. **CFIA approved 2017.**
3.2	2017-01-20	Stephen Hill	Minor changes, added in clean / dirty images.
3.3	2017-04-12	Stephen Hill	Small edits and clarifications. Changing demand for full change of PPE to use of secondary PPE in wet labs (elaborated in CDRF.B.2010). Added note on work in multiple facilities.
3.4	2019-03-11	Stephen Hill	Approved all changes (accepted from CFIA in 2018). Added notes on sign-in procedures, minor edits.

Department of Ocean Sciences
Ocean Sciences Centre
Cold-water Deep-sea Research Facility

Containment zone entry and exit procedures for large items and equipment

Version	Date of last revision
3.0	2019-03-11

Description

Entry to the containment zone is limited to authorized personnel and normally occurs through the entry and exit anterooms (CD-1012 / CD-1013 and CD-1014 / CD-1016 respectively). Items which may not reasonably pass through and be decontaminated in the personnel anterooms rooms must pass through the containment barrier from a different route. This may include large pieces of equipment, fish tanks, bulk feed or reagents, etc. Because large items may not easily fit through the interlocked entrance, a temporary exception to full biocontainment protocols must be arranged. Contact the facility manager or containment technician to organize this. Since this means direct entry to the containment zone (without the use of an interlocked corridor), the process requires decontaminating the receiving room and transported items before handing them through the containment barrier. These controls have the primary aim of preventing the release of infectious material or invasive species outside of the lab. The secondary goal is to prevent pathogens from being carried into the containment zone where they may contaminate ongoing experiments.

See the following SOPs for other types of entry and exit protocols.

For personnel and small items refer to SOP *CDRF.B.1010.*

For movement of animals refer to SOP *CDRF.A.7010.*

For transport of pathogens refer to SOP *CDRF.B.3010.*

Glossary and terms

- **BSO: Biosafety officer**
- **CZ: Containment zone**
- **PPE: Personal protective equipment**
- **SOP: Standard operating procedure**
- **RA: Research assistant**

Safety and responsibilities.

- **All personnel must first obtain access permission from the facility manager as detailed in the biosafety manual introductory material (conditions of access, roles and responsibilities).**

- **Training in SOPs on personal protective equipment and habits (CDRF.B.2010).**
- **Wear all suitable PPE (lab coat, gloves, CZ footwear).**
- **Lift heavy equipment with more than one person.**

Protocols

This is the preferred way to bring large items into the CZ, in order of preference:

1. **Remove packaging, break down large items or transport groups of animals in smaller containers until the delivery is small enough to enter through the interlocked anterooms.**
2. **Large items may pass through the invasive species room (CD-1011) or either of the disease challenge doors (CD-1017 A or B) if any of these rooms is not currently housing animals and has been decontaminated.**
3. **Otherwise, other items destined for the dry labs and interior should pass through the invasives room (CD-1011) provided it is not being used for pathogen work.**
   This is also the only room with double doors passing through to the interior. This includes rooms CD-1022, 1023, 1025, 1030 and the effluent treatment room CD-1026.

Entering

1. **Notify the containment RA or facility manager of the arrival of large materials that need to be brought into the CZ. They will inform all personnel working in the CZ.**
   Complete the fish transfer form if moving animals. Schedule this in advance.
2. **All work in the entrance room should stop. Interior doors of the access room should remain closed.**
3. **Decontaminate the receiving room.**
   Transport of large items is best arranged before beginning a new experiment, since the room will already have been decontaminated. If the transport is happening during an experimental run, decontaminate the floors and the interior surface of the doors to be opened.
4. **Before passage into the CZ, remove outer, absorbent packaging (i.e. cardboard) as well as any other packaging that may cover non-sterile surfaces.**
5. **If items have visible dirt on them, first wash with soap and water and rinse.**
   Hoses in the deep-sea room (CD-1002) may be used to wash off items used in the field. This room has drains to receive water.
6. **Don gloves and decontaminate all outer surfaces with Virkon Aquatic spray at 1:100 dilution.**
   This includes carts and cart wheels. Decontamination supplies can be found by the CZ entrance. Virkon is a good general disinfectant. Alternatives, such as bleach or alcohol may be appropriate in some cases. See SOPs B.4020 and B.4010 for more information on preparation and selection of decontamination chemicals.

7. **Allow 15 – 20 min to pass with the solution in contact with surfaces. Reapply as necessary during this time.**
8. **Rinse off with warm freshwater and allow to dry.**
9. **Open the door, pass materials to staff inside the facility or place across the threshold.**
   If the positive pressure alarm is on, do not enter.
10. **Immediately close the door. Do not prop open or otherwise leave the door open.**
    If personnel need to cross the barrier, they should be wearing proper PPE. After crossing, they should exit through the anterooms as soon as possible following the normal exiting SOP.

Exiting

1. **Reverse the procedures of the above.**
2. **Notify staff in the CZ and stop work in the egress room. Bring the items into the egress room and close all doors to this room.**
3. **Decontaminate all surfaces as above using decontamination supplies from within the CZ. If items are small enough to bag, do so.**
   Equipment such as gas diffusers, electrical monitoring equipment and other delicate or porous items should be removed for individual cleaning, disinfection or replacement. Fluids will pass into the drains which all lead to effluent treatment.
4. **Open the door, pass materials to staff outside the CZ or place across the threshold.**
5. **Immediately close the door. Do not prop open or otherwise leave the door open.**

Materials

- Scrubbing brushes
- Household soap for cleaning
- Virkon Aquatic (available from OSC stores) or other suitable decontamination solution

References

1. *Operational Procedures Manual - Decontamination (Version 1.0) in Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN)*. (Australian Department of Agriculture, Fisheries and Forestry, 2008). at <http://www.daff.gov.au/__data/assets/pdf_file/0008/617183/decontamination-manual.pdf>

2. Canadian Biosafety Standards, 2nd edition (2015).

3. *Containment Standards for Facilities Handling Aquatic Animal Pathogens*. (Canadian Food Inspection Agency, Biohazard Containment and Safety Science Branch, 2010).

4. *Laboratory biosafety manual*. (World Health Organization, 2004).

Version history and notes

Version	Date	Authors	Notes
1.0	2014-05-14	Stephen Hill	Created. Based on a review of CFIA and PHAC biosafety manuals[1–4].
1.1	2014-06-26	Stephen Hill	Edits from KG, MR
2.0	2014-07-23	Stephen Hill	Edits based on first CFIA review. **CFIA approved 2017.**
2.1	2018-04-12	Stephen Hill	Made more restrictive. Pre- 2018 CFIA submission.
3.0	2019-03-11	Stephen Hill	Approved by CFIA in 2018. Minor edits.

Memorial University of Newfoundland
Department of Ocean Sciences
Cold-ocean Deep-sea Research Facility

Scheduled housekeeping and maintenance in the containment zone

Version	Date of last revision
3.1	2019-10-23

Description

The following are routine items that should be performed by the containment zone Research Assistant (CZ RA) or facility manager.

Glossary

- **BSC: Biological safety cabinet**
- **CZ: Containment zone**
- **WWTP: Wastewater treatment plant**

Beginning and end of day

1. **Confirm that there is inward directional airflow and negative pressure.**
   This is also done on each entry and exit. If negative pressure is not being maintained throughout the day, speak to Facilities Management who may adjust the air handling systems.
2. **Make rounds on animals and tanks.**
   Look for escapes, mortalities and signs of distress and illness as per the animal care protocols for that experiment. Record water quality parameters if part of project. Look for leaks, pump and power failures or malfunctioning equipment.
3. **Replace footdip Virkon as needed.**
   Replace when it loses its pink colour or the volume is too low. Note in room records.
4. **Replace net dip bleach.**
   Replace when solution becomes fouled or top up with new solutions. Do not leave completely unrefreshed for more than 3 weeks. Note in room records.
5. **Top up low or empty ethanol spray bottles (70%).**
6. **Inspect the WWTP.**
   Check for low level and UV failure alarms. Check for leaks, pump and power failures or malfunctioning equipment.
7. **Wipe UV bulbs in WWTP and CD1010.**
8. **Inspect incubators.**
   Check temperature and gas levels.

9. **Tidy and disinfect all actively used work surfaces. Collect full garbage for weekly autoclaving.**

Weekly

1. **Check mounted and handheld water quality monitors for calibration.**
2. **Check autoclave drain traps. Clean autoclave as necessary.**
3. **Perform a biological indicator test on waste loads for each autoclave on first available load.**
4. **Empty waste and sterilize.**
5. **Check status of lab water systems. Replace expired consumables.**
6. **Run water in all sinks to maintain water traps. Check that toilets are full.**
7. **Clean all stainless steel counters.**
   Warm water or soap and water is sufficient. Do not use abrasive cleaners. Clean counter tops as well as cabinet and frame faces.
8. **Check liquid nitrogen levels in CD-1022 dewar.**

Monthly

1. **Clean filters on -80°C freezers and chillers.**
2. **Check operation of the intercom system.**
3. **Check operation on emergency oxygen and alarm systems on tank monitoring sensors.**

Annually

1. **Certification of BSCs.**
2. **Certification of fume hoods.**
3. **Certification of backflow preventers.**
4. **Full smoke test of CZ penetrations.**
5. **Verification of door interlocks.**
6. **Check of WWTP glass media filters. Replace media as necessary.**
7. **Check and replacement of WWTP drum filter screens as necessary.**
8. **Confirmation of system operation in power outage while under generator power (or whenever power outage occurs).**
9. **Preventative maintenance of autoclaves.**
10. **Review door signage and contact details.**
11. **Smoke test of barrier penetrations.**
12. **Test CZ intercoms and phones.**

Version history and notes

Version	Authors	Notes
1.0	Stephen Hill	First writing.
1.1	Stephen Hill	Added in post-inspection details. **CFIA approved 2017.**
1.2	Stephen Hill	Minor changes. Pre CFIA renewal.
3.0	Stephen Hill	Reviewed and approved in 2018 by CFIA. Minor edits 2019.
3.1	Stephen Hill	Added several items. APPROVED 2020-10-09

Biocontainment personal protective equipment (PPE)

Version	Date of last revision
3.0	2019-03-21

Description

Everyone entering the CDRF containment zone must wear appropriate protective clothing and equipment. These controls have the primary aim of protecting the health and safety of the operator. Correct use of PPE in the containment zone (CZ) will also play an important part in preventing the release of infectious material or invasive species from the CZ or from carrying such materials into the CZ where they may contaminate ongoing experiments. Within the CZ, minimal PPE includes lab coats or other suitable outerwear, gloves when handling infectious material or exposed equipment and dedicated footwear or foot coverings. Additional safety ware may be required, including safety glasses or masks, waterproof coverings and respirators as described in task-specific SOPs. These procedures apply to all persons entering the containment zone, including guests and janitorial or maintenance staff.

Glossary

- **BSO: Biosafety officer**
- **CZ: Containment zone**
- **JBARB: Joe Brown Aquatic Research Building**
- **PI: Principal investigator**
- **PPE: Personal protective equipment**
- **RA: Research assistant**
- **SOP: Standard operating procedure**

Safety and responsibilities.

- **The containment RA will order and maintain common use PPE. This includes decontaminating used items as necessary.**
- **The PI, with the assistance of the facility manager and BSO, must assess the risks of a new project to determine what PPE is required.**
- **It is the responsibility of all lab users to become familiar with the correct use of PPE and to follow the procedures correctly. Failure to use PPE when required will result in retraining and possible exclusion from the CDRF.**
- **Make sure all PPE fits properly and is in good condition before using. Check for tears or worn seals in waterproof boots and bibs.**

Preparations

Certain items of PPE are required at all times as indicated below. Others, such as facemasks and waterproof clothing are task dependent. A risk assessment conducted for new procedures will identify required PPE.

Protocol

Primary PPE

Don these items of PPE when entering the CZ at the change lockers. Obtain correct CZ PPE from the CZ RA.

1. **Lab coats or scrubs are required.**
   Fully button the lab coat. Lab coats should have an elastic cuff. Disposable lab coats are available for guests but regular lab workers should not use them.
2. **Latex or nitrile gloves are required.**
   Do not reuse gloves. Dispose of them in biohazard waste.
3. **Wear safety goggles or a face shield when there is a risk of aerosol generation, splashing or any exposure to the eyes from infectious or harmful material.**
   As indicated in task-specific SOPs. This includes handling live animals, boiling fluids, opening sealed containers outside of the BSC, etc.
4. **Don rubber boots or water resistant footwear. Footwear must completely cover the foot and be non-slip. Tuck pants into boots.**
   Provide guests with disposable boot covers. Footbath fluid should make full contact with the sole of the boot or shoe when immersed. Discarded disposable covers in a biohazard waste container after use. Staff doing lab inspections or maintenance work and not engaging in research activities (e.g. facilities service technicians) may transit the space in their regular work boots provided they immerse them in the footbath.
5. **Do not exit the facility wearing PPE from the CZ. Do not enter other aquatic facilities, such as JBARB, with clothing and footwear that was not covered while in the CDRF CZ.**
   Take extreme caution if working in the CDRF and other aquatic facilities on the same day. Avoid this if possible or schedule such that work in the CZ is the last activity of the day.
6. **Used lab coats and scrubs that require cleaning should be autoclaved before the user cleans them.**
   Laundering performed off-site.

Secondary PPE

In addition to the primary PPE listed above, add secondary PPE when performing specific tasks as listed in the table below.

Activity / PPE	Lab coat	Safety glasses	Inner gloves	Outer gloves	Lab shoes	Tall rubber boots	Apron	Rain gear	Face shield	Surgical mask	Scrubs	Waterproof sleeves
Entry to dry lab	●	○	●		●							
Work with infectious materials	●	●	●	●	●				○	○		○
Entry to wet lab	●	●	●			●						
Wet lab hosing down tanks / room			●	●		●		●	●			
Surgery	●	●	●	●		●	●			●		●
Necropsy	●	●	●	●		●	●			○		●
High risk of contamination from splashing		●	●	●		●	●	○	○		●	○

Recommend PPE for selected activities. ● Mandatory ○ Optional, as required

Materials

Supplier	Catalog #	Item Description
Fisher Scientific	19 166 224	LAB COAT SMALL
Fisher Scientific	19 166 225	LAB COAT MED
Fisher Scientific	19 166 226	LAB COAT LARGE
Fisher Scientific	19 166 227	LAB COAT XLARGE
U-Line	S-19250	Waterproof Boot Covers
Fisher Scientific	19-181-529	Disposable Polyethylene Aprons
Fisher Scientific	19-170-904	Polyethylene sleeve covers

References

1. *Laboratory biosafety manual*. (World Health Organization, 2004).

2. *Canadian Biosafety Standards and Guidelines*. (Government of Canada, 2015).

3. *Containment Standards for Facilities Handling Aquatic Animal Pathogens*. (Canadian Food Inspection Agency, Biohazard Containment and Safety Science Branch, 2010).

4. Pacific Region Animal Care Committee, Management Procedure 1.2, Pacific Biological Station Biosafety Procedures

Version history and notes

Version	Date	Authors	Notes
1.0		Stephen Hill	First version. Based on review of WHO, CFIA and PHAC biosafety manuals[1–3] as well as samples from other aquatic animal biocontainment facilities.
1.1	2014-06-26	Stephen Hill	Edits from KG, MR. CFIA first submission.
2.1	2016-12-19	Stephen Hill	Changed laundering instructions. Removed DRAFT status. **CFIA approved 2017.**
2.2	2018-04-16	Stephen Hill	Separation into primary and secondary PPE. Not longer require full change of PPE when conducting work in wet lab, rather will cover primary PPE with waterproof materials. Addition of secondary PPE to materials.
3.0	2019-03-21	Stephen Hill	Review and minor edits.

Movement, transport and storage of infectious materials

Version	Date of last revision
3.0	2019-03-21

Description

Each new project involving materials that may contain pathogens begins with the transport of these materials into the containment zone or movement within it. The institutional biosafety committee (IBC) must first approve the use of pathogens before they enter the CDRF.

All pathogens should be stored within the containment zone of the CDRF. Pathogens may be received in many forms such as lyophilized powder, frozen stocks, liquid cultures, slants, cell culture, infected tissue, contaminated water or liquids, etc. It is preferable to receive pathogens as a validated stock culture in lyophilized form from a supplier such as the American Type Culture Collection (ATCC). Correct receipt of pathogens will prevent accidental exposure and misplacement of the pathogen.

During movement or transport, there is an enhanced risk of contamination due to spills or breakage from containers. Importation or transport of aquatic pathogens within, into and out of Canada is regulated by Transport Canada through the Transport of Dangerous Goods Regulations. Permits to acquire aquatic pathogens from outside of Canada are obtained from the Canadian Food Inspection Agency. Permitting for importing terrestrial and human pathogens is through the Public Health Agency of Canada (contact the institutional BSO, for more information). Unless transport within Canada is specifically restricted, further CFIA / PHAC permits are not required to transport within Canada so long as the receiving lab is certified at the containment level of the infectious material. A “Biohazardous Agent Transfer Notification form” which can be found in Appendix 6 of MUN’s Biological Safety Manual must be completed by both supplier and recipient prior to transfer. Forward a copy of the completed form to MUN’s BSO.

These protocols detail aspects specific to movement and transport of pathogens. Due to the potential regulatory complexity of the issue, please see the links in References for more specific information.

Glossary

- **BSC: Biosafety cabinet**
- **BSO: Biological safety officer**
- **CFIA: Canadian Food Inspection Agency**
- **CZ: Containment zone**
- **IBC: Institutional biosafety committee**
- **MSDS: Material safety data sheet**

- **EHS: Environmental Health and Safety**
- **PHAC: Public Health Agency of Canada**
- **TDGR: Transportation of dangerous goods regulations**

Safety and responsibilities.

- **Approval for use of the pathogen must be obtained from the IBC, listing the CDRF as a site of use.**
- **CDRF staff and student supervisors (or delegate) must train all users handling pathogens in biosafety techniques and protocols.**
- **All personnel should be aware of the pathogens in use in the lab and have read the pathogen safety data sheets and / or MSDSs for them.**
- **In the majority of cases, manipulate pathogens in the BSCs. If there is any risk of generating aerosols, use a BSC.**
- **The principal investigator, with the assistance of the BSO, is responsible for obtaining the correct permits to import pathogens from outside of Canada into the CDRF.**
- **Lab users must notify the containment RA and BSO of any plans to move or transport pathogens and to correctly inventory the relocation. Please see MUN's Biological Safety Manual for more information on movement and transport requirements.**
- **The facility manager is responsible for the maintenance of records on pathogen relocation and storage. These will be audited by the institutional BSO.**
- **If you are moving pathogens yourself, you must have TDGR training. Rod Hobbs (phone 709-864-8250) is the OHS officer with TDGR training. Contact him for assistance with TDGR training.**

Protocols

Receiving and inventory of infectious material

1. **Pathogens are not allowed into the lab unless a pathogen safety data sheet is available and they are listed on the current MUN biosafety certificate.**
   The facility manager and BSO can assist principal investigators in completing a risk assessment and coordinate with the CFIA and IBC.
2. **Samples with unknown pathogenic potential should be treated according to the risk from the highest possible category of pathogen.**
   In the case of material such as diseased tissue or contaminated water where the precise pathogenic content may not be known, a risk assessment should be completed indicating a containment level and practices appropriate to the pathogen of greatest risk that the sample may contain. This also must be approved by the IBC
3. **Follow SOPs *CDRF.B.1010* or *CDRF.A.7010* for receiving items into the CZ.**
   For contained samples and animals respectively.

4. **Decontaminate the exterior surfaces of containers and open outer containers in the BSC. Check that the samples have not spilled or broken and are properly labelled.**
   Choose an appropriate decontamination method (SOP CDRF.B.4010). Generally, 70% ethanol spray is appropriate.
5. **Follow the pathogen-specific SOPs at this time if stock or working sub-cultures are to be made; otherwise label and store the pathogen as described below.**

Storage and inventory of infectious material

Accurate and updated records of the location of all infectious material must be kept. The location of all pathogens and pathogenic material must be tracked within the facility. Short term use (use within 3 weeks), such as sub-culturing for experimentation, does not require inventorying, although proper labelling is required. Follow these rules when storing pathogens.

1. **Tubes and boxes to be labelled clearly with the deposition date, project code, genus, species and strain details of the pathogen. This includes working cultures, plates, flasks, etc.**
2. **Containers and freezers holding pathogens to be marked with "biohazard" tape or signage.**
3. **Long term storage conditions must prevent further growth of the organism, i.e. frozen or lyophilized.**
4. **Short-term storage should be limited to no more than 3 weeks at 4°C. After this time, move pathogens to long-term storage and the long-term inventory.**
5. **Long term storage is in the -80°C freezer in room CD-1022. Use the provided racks and boxes.**
   These hold up to 2 ml centrifuge tubes. Avoid using larger sized containers if not necessary and always ensure that the container is safe at -80°C.
6. **If there is a need to store pathogens outside of the containment zone, first notify the BSO. Such material must be stored in leak proof containers in a locked room or cabinet with restricted access.**
   This location should be indicated in either the CDRF inventory or in the inventory of the new location provided it is allowed under the biosafety certificate.

Inventory and access

Inventory all items entering long-term storage. This is a requirement of certification and prevents accidental release, loss or disposal of pathogens. Paper records include a usage log for each pathogen detailing their use and disposal. It is the responsibility of the containment RA to keep records up to date.

Adding to the inventory records

1. **Determine the storage location and conditions for your sample.**
2. **From the inventory binder at this location, turn to the section appropriate for the sample type: bacterial, viral, parasite, cell line or animal (whole or tissue) and other.**

3. **Fill out a blank entry form with as much information as is available as well as the precise storage location.**
   This includes enough information to be able to identify the organism at the finest level known. E.g. bacterial genus, species and strain information should be given.

   Samples which are believed to contain unknown pathogens should be labelled with the source of the material as well as the AQC level as determined by a risk assessment.
4. **Multiple aliquots / samples of the same pathogenic material can be recorded on the same sheet indicating the multiple storage sublocations (i.e. box wells) in that field.**

Removal from inventory for use and disposal

5. **For each pathogen record sheet there is a log section. Locate the sheets for the pathogens to be used and complete the fields listed there.**
6. **If the pathogens are being removed for disposal, indicate the disposal method and mark the disposal date in the main section of the record. Move the record to the "discarded" section of the binder.**

Movement within the containment zone and within the CDRF

In general, safe movement of pathogens within the containment zone (e.g. from freezer to BSC) simply depends on attentive following of biosafety practices, with emphasis on the following:

7. **Use sealed, labelled and closed leak-proof containers. Threaded screw-top tubes are preferable to snap-top tubes.**
8. **If the movement is between buildings, the material must be in an impact-resistant container.**
   You can borrow appropriate containers from the CDRF. For cold samples, place ice in a secondary, labelled outer container. Allow to vent if dry ice is used.
9. **Use a cart with side rails if larger quantities of infectious material are being moved.**
10. **Have a spill kit readily available for the quantity of material you are moving.**
11. **Notify the facility manager or containment RA before bringing infectious material into the CDRF.**
    This material must be listed on a current biosafety certificate, which will be confirmed by the facility manager.
12. **Inventory and store the new entry as above.**

Transport between buildings and shipping in Canada

Please see section 7 of the MUN Biosafety Manual for detailed instructions on transport.

If infectious material is being moved between buildings at the Ocean Sciences Centre or from the main campus of Memorial University, special packaging to contain spills in the event of breakage must be used as per the TDGR. For most pathogens, this will mean type 1A packaging. This packaging may be loaned from the CDRF. The same packaging is used for shipments within Canada.

- **The receiving lab must have certification from the appropriate agency and at the required level.**

Shipping Infectious Substances

A Type 1A container is a triple packaging system consisting of:

- watertight primary receptacle(s);
- a watertight secondary packaging;
- absorbent material; and
- an outer packaging.

Type 1A packaging

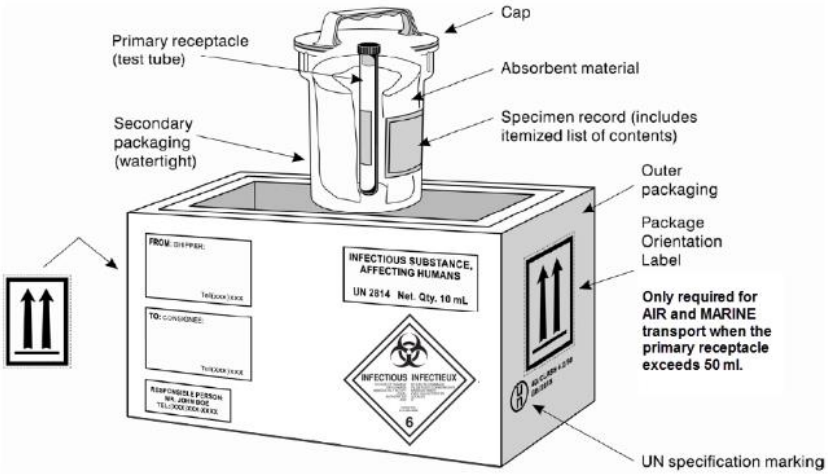


Figure 1: Example of triple packaging system for the packaging and labelling of Category A infectious substances (*Figure provided by IATA, Montreal, Canada*)

Materials

A variety of category A shippers are available from SafTPak
http://www.saftpak.com/STPPack/ProductDetail.aspx?ID=192

References

Transportation of Dangerous Goods

http://www.tc.gc.ca/media/documents/tdg-eng/RDIMS-8210418-SHIPPING_INFECTIOUS_SUBSTANCES_-_TDG_BULLETIN_FINAL.pdf

Import permits

http://www.phac-aspc.gc.ca/lab-bio/permits/imp-permit/index-eng.php

http://www.inspection.gc.ca/animals/aquatic-animals/imports/pathogens/eng/1312436244596/1322885037191

1. *Laboratory biosafety manual*. (World Health Organization, 2004).

2. *Canadian Biosafety Standards*. (Government of Canada, 2015).

3. *Containment Standards for Facilities Handling Aquatic Animal Pathogens*. (Canadian Food Inspection Agency, Biohazard Containment and Safety Science Branch, 2010).

Version history and notes

Version	Date	Author	Notes
1.0	2014-03-18	Stephen Hill	First writing. Based on above links and the CBSG, WHO and CFIA biosafety manuals[1–3].
1.1	2014-05-14	SH, after comments from Matt Rise, Kurt Gamperl	Mainly minor edits – clarification on what to record for different types of inventory.
2.0	2014-07-24	Stephen Hill	After first CFIA review. Added clarification on permitting to certified lab and off-site storage.
2.1	2015-03-12	Stephen Hill	After review by Rod Hobbs. Adding university requirement.
2.2	2015-05-05	Stephen Hill	Minor edits. Pre-submission. **CFIA approved 2017.**
2.3	2018-04-16	Stephen Hill	Removed references to digital pathogen inventory records, will just use paper.
3.0	2019-03-21	Stephen Hill	Some clarifications of TDGR responsibilities, other minor edits.

Memorial University of Newfoundland
Department of Ocean Sciences
Cold-Ocean Deep-Sea Research Facility

CDRF.B.4010

General decontamination and disinfection

Version	Date of last revision
3.0	2019-03-21

Description

Decontamination is fundamental to preventing the spread of pathogens within and outside of the lab. All waste must be decontaminated before disposal, and decontamination of surfaces and equipment is required to protect the user and to avoid experimental cross-contamination. Decontamination includes the use of autoclaves and chemical disinfectants. The following focuses primarily on chemical disinfectants used in the dry labs and in more general settings. For sterilization of waste and the autoclave, as well as particular methods for the wet labs, see SOPs:

For sterilization, biohazard waste and the autoclave refer to SOP *CDRF.B.6010.*

For necropsy lab decontamination refer to SOP *CDRF.B.4020.*

For general information on laboratory disinfection, pleas also see the MUN Biosafety manual *BSOP-03*.

The selection of an appropriate disinfectant will depend upon the organism in use. The application of the disinfectant requires consideration of factors such as organic load, concentration and contact time, among others. A risk assessment must be done when any new pathogens are introduced to the lab, and this protocol revised. Details on the selection of appropriate disinfectants are in chapter 16 of the CBSG.

Presently, two general disinfectants are used in the lab: chlorine (bleach) and alcohol. Additionally, specialized disinfectants are used in the wet labs and footbaths as discussed in SOP *CDRF.B.4020*. Used properly, these disinfectants are effective against most fungal spores, vegetative Gram positive and Gram negative bacteria, enveloped viruses and mycoplasma. If your materials potentially contain infectious prions, protozoal oocysts, bacterial endospores, Mycobacteria or non-enveloped viruses, other disinfectants may need to be used. First consult the BSO before introducing such organisms.

Glossary

- **BSO: Biosafety officer**
- **CBSG: Canadian Biosafety Standards and Guidelines**
- **CZ: Containment zone**
- **EtOH: Ethanol**
- **OSC: Ocean Sciences Centre**
- **PPE: Personal protective equipment**
- **RA: Research assistant**
- **SOP: Standard operating procedure**

Safety and responsibilities.

- **All users of the facility must be familiar and trained in these SOPs.**
- **Correct PPE should be used. Note, bleach is corrosive – use bibs for large quantities to protect clothing.**
- **Users are responsible for decontaminating all surfaces and, as necessary, equipment before and after use. This includes lab counters, BSCs, pipettes, etc.**
- **Users should replenish disinfectant solutions from stock as they use them. The containment RA is responsible for maintaining these stocks and working solutions.**
- **The principal investigators must identify which pathogens will be used to the BSO and select appropriate disinfectants as part of a risk assessment and as outlined in the biosafety certificate application.**
- **Janitorial staff are allowed access to the CZ for cleaning of floors and washrooms when there are biosafety trained staff available to supervise, but not the disease challenge or invasive species rooms. Janitorial supplies are kept within the CZ.**
- **Staff or students that are running experiments are responsible for cleaning before and after an experiment. This includes both dry and wet labs. The containment RA will verify that this is done correctly.**

Preparations

1. **Before beginning work, make sure appropriate disinfectant is available.**
2. **Disinfect all work surfaces before beginning work and after concluding it.**
3. **70% ethanol has long term stability, so long as it is in a closed container that does not allow evaporation.**
4. **Working bleach solutions are unstable especially if in contact with light. They should be kept in closed, dark containers and made fresh. Do not keep for longer than a month.**
5. **Label all solutions with contents and preparation date.**

Protocols

1. **If the surface you are decontaminating is visibly dirty, first clean with detergent to remove dirt.**
2. **Thoroughly spray the surface with the disinfectant and allow for an appropriate contact time.**
   This is short, about 1 min, for susceptible organisms or long, about 10 min, for resistant organisms.
   a. **70% ethanol: Good for general use.**
      Evaporates quickly, so is not suitable for long contact times. Avoid using 100% ethanol, which evaporates too quickly and does not effectively penetrate cells. Diluted in pure (type 2, Elix) water.

b. **Chlorine (bleach): Should contain 0.5% free chlorine. Remove after contact by wiping down with 70% EtOH or sterile water. Must be kept in dark.**
Chlorine is corrosive to metals, so must be cleaned off after use. Can be made from commercial bleaches, such as Clorox, which contains 5.84% available chlorine, so a 10X dilution in water is used. Should be kept in opaque bottles and refreshed frequently. Is generally more effective than EtOH, including for the decontamination of DNA.

3. **For highly contaminated surfaces or where cross-contamination is a particular concern, first use bleach and then 70% EtOH.**

Materials

- Commercial bleach, such as Clorox.
- Spray bottles. Dark bottles for bleach.
- Ethanol from OSC stores.

References

1. *Laboratory biosafety manual*. (World Health Organization, 2004).

2. *Containment Standards for Facilities Handling Aquatic Animal Pathogens*. (Canadian Food Inspection Agency, Biohazard Containment and Safety Science Branch, 2010).

3. *Canadian Biosafety Standards and Guidelines*. (Government of Canada, 2015).

Version history and notes

Version	Date	Authors	Notes
1.0	2014-03-05	Stephen Hill	First writing, largely based on CBSG and other guidelines[1–3].
1.1	2014-06-27	Stephen	Minor edits, CFIA first submission. **CFIA approved 2017.**
2.0	2018-05-03	Stephen Hill	Minor edits. Pre 2018 CFIA resubmission. Added reference to BSOP-03.
3.0	2019-03-21	Stephen Hill	**Approved 2018.** Minor edits.

Memorial University of Newfoundland
Department of Ocean Sciences
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CDRF.B.4020

Cleaning and disinfection – necropsy and wet labs

Version	Date of last revision
3.0	2019-03-21

Description

To prevent contamination and/or cross-contamination it is imperative to completely and effectively clean, disinfect and prepare for re-use, all areas in wet labs after the handling of fish. This includes all surfaces of tanks, floors and necropsy counters as well as nets or other equipment used to handle fish. Although janitorial staff will clean the floors in other areas of the containment zone, they are not allowed in the necropsy labs. Selection of an appropriate disinfectant will depend on the pathogens present, or potentially present. Appendix 1 lists common disinfectants and their range of action. For each new pathogen, disinfection procedures will be considered as part of the risk assessment. Virkon is effective for most applications, including ISAV and Nodavirus challenges and its use is described here.

Glossary

- **BSO: Biosafety officer**
- **CZ: Containment zone**
- **PPE: Personal protective equipment**
- **RA: Research assistant**
- **SOP: Standard operating procedure**
- **WHMIS: Workplace hazardous materials information system**

Safety and responsibilities.

- **This procedure applies to all principle investigators, research assistants/technicians and students who use the CDRF necropsy wet labs and containment laboratories.**
- **All room and tank start-up and tear-down decontamination is to be recorded in the animal care / experiment log.**
- **All users handling chemicals must be aware of WHMIS requirements.**
- **PPE required: Latex or nitrile gloves, rubber boots, safety glasses, lab coat, waterproof bibbed pants or apron.**

General Information

Keep work area clean and tidy.

Keep equipment, tanks, covers, project separators (dividers), etc. in good condition, rust-free, and corrosion-free.

Clean tanks regularly to prevent an accumulation of organic matter.

Clean, disinfect and dry tanks prior to housing different groups of fish.

Each disease challenge (CD-1017 A/B) and invasive species (CD-1011) tank room has designated equipment (e.g. cleaning brushes, buckets, etc.) that should not be shared with other rooms.

Each tank within a room has designated equipment (e.g. dip nets) that should not be used in other tanks within the room.

After use, equipment such as dip nets, air-stones, buckets and feeding equipment will be cleaned, disinfected, dried and put away in the proper place.

Type of chemical disinfectant to be determined by the primary investigator in consultation with the BSO and containment RA in accordance with the Pathogen Risk Assessment (see Appendix 1 for summary of common disinfectant types).

Products are used according to manufacturers' directions.

Remove organic matter from equipment prior to disinfection to ensure efficacy.

Maintain disinfectant concentrations by checking concentration (e.g. test strips or pH check) or regular renewal of the product (e.g. net dips & footbaths replaced weekly).

Dispose of disinfectants according to manufacturer directions and the requirements of waste management regulations.

Protocols

Tank decontamination (CD-1017 A/B, CD-1011)

1. **All fish stocks, carcases, faecal matter and uneaten feed should be removed from tanks for disposal.**
   See protocol CDRF.B.6010 for waste disposal.
2. **Drain tanks and associated pipes, taking into account the safe disposal of contaminated water.**
3. **Any ancillary equipment, such as feeders, aerators or lights, should be removed for separate cleaning and disinfection.**
4. **Tank surfaces should be sprayed/washed to remove any gross fouling.**
   As of this writing, do not use large amounts of freshwater, which is coloured and may occlude the UV lights in effluent treatment. Wash with seawater and do quick rinses with fresh.

5. **Spray detergent onto surfaces and then wash the area by scrubbing. If possible, heated water should be used to enhance the cleaning process.**
   A 1:200 dilution of Virkon Aquatic prepared in freshwater may be used as detergent. Alternatively household detergent may be used. Wash from the top down.
6. **Rinse with warm water.**
   Allow excess water to drain away before disinfection step.
7. **Spray disinfectant onto surfaces**
   Use 1:100 Virkon Aquatic prepared in freshwater for disinfection (see product information in Appendix). The application of disinfectants should start from the base of the tank and proceed upwards.
8. **Allow the disinfectant to remain in contact with the tank for 15-20 min.**
   When applying disinfectants to vertical surfaces, care must be taken to ensure that adequate contact time is maintained before the disinfectant drains away. Reapply as needed.
9. **Rinse with clean freshwater and allowed to dry completely.**
10. **All tanks should remain dry until the room is ready for restocking.**

Floor Cleaning/Disinfection

Following tank disinfection, it will be necessary to follow room/floor disinfection protocols. Floor drain troughs serve as tank drain receptacles and water flows to the wastewater treatment plant.

1. **Clean the floor by sweeping away any solid debris.**
2. **Hose floor off with and use a squeegee to push water into a floor drain.**
3. **If required due to heavy soiling, clean the floor with a suitable detergent prior to disinfecting.**
   1:200 Virkon Aquatic or household detergent may be used.
4. **Apply disinfectant to the complete floor surface area and let sit for 15-20 min.**
   Use 1:100 Virkon Aquatic prepared in freshwater for disinfection (see product information in Appendix).
5. **Following disinfection, rinse floors with freshwater.**
   Any excess water build up on the floor should be pushed into the floor drain with a squeegee and the floor allowed to dry completely.

Foot Baths

Footbaths should be placed at points of entry and exit to reduce tracking of contamination from within or between infected areas.

Footbaths are found at the boundary of the containment area (e.g. at the entry of CDRF) and before the necropsy (CD-1017 A, B) and invasive species rooms (CD-1011).

Footbaths should be large enough to allow the person to stand with both feet in the solution, and deep enough to cover outsoles.

Disinfectant in footbaths should be drained and refreshed at least weekly and a log kept of changes of the disinfection solution.

For Virkon Aquatic disinfectant footbaths

1. **Prepare 1% Virkon (1:100) in freshwater (as per product information in Appendix).**
2. **Place Virkon solution in non-slip footbath tray at entrance/exit of wet labs.**
3. **Replace/replenish every 4 days or as needed (Virkon solution is stable up to 7 days).**
   Replace Virkon if colour changes from pink to light pink or milky.

Net Dips

Use a container deep enough immerse the net fully.

1. **Fill container with a 0.5% free chlorine bleach solution.**
   Should be refreshed once a week or as needed. Logs are attached to each tank.
2. **Place net in the dip container. Leave net soaking in solution for 15-30 minutes.**
3. **Remove from dip, thoroughly rinse with clean water and hang to air dry.**

Pass-through

A stainless steel pass-through box connects CD-1017 B to the corridor. This may be used to pass specimens into the necropsy room. Infectious samples must be transported properly and the pass-through decontaminated after each use (from the necropsy side) as detailed in SOPs CDRF.B.3010 (Transport) and 4010 (Decontamination) respectively.

Materials

- Washing supplies: scrubbers, squeegees, hoses.
- Virkon Aquatic or other decontamination solution and detergents.

References

Canadian Council on Animal Care Guidelines on: the care and use of fish in research, teaching and testing. (http://www.ccac.ca/Documents/Standards/Guidelines/Fish.pdf)

World Organization for Animal Health: Manual of Diagnostic Tests for Aquatic Animals 2013. Chapter 1.1.3 Methods for Disinfection of Aquaculture Establishments. (http://www.oie.int/fileadmin/Home/eng/Health_standards/aahm/current/1.1.03_DISINFECTION.pdf)

DFO Pacific Region Animal Care Committee Management Procedure 2.6, Revision 1. Pacific Biological Station Equipment Cleaning and Disinfection Procedures.

General disinfection guidelines. 1995. R.F. Kahrs. Rev. Sci. Tech. Off. Int. Epiz. 14(1):105-122.

Torgersen Y and Hastein T (1995). Disinfection in aquaculture. World Organisation for Animal Health, *Scientific and Technical Review* 14(2), June 1995, Paris.

Quinn PJ and Markey BK (2001). Disinfection and disease prevention in veterinary medicine. In: *Disinfection, Sterilization and Preservation*, 5th edition, Block SS (ed), Lippincott Williams & Wilkins, London.

Department of Agriculture, Fisheries and Forestry (2008). Operational Procedures Manual — Decontamination (Version 1.0). In: Australian Aquatic Veterinary Emergency Plan (AQUAVETPLAN), Australian Government Department of Agriculture, Fisheries and Forestry, Canberra, ACT.

Appendix 1: Disinfectants

Appendix 1: Characteristics of Common Disinfectants				
	Iodophors	*Hypochlorites*	*Oxidizers*	*Quaternary ammonium compounds*
Brand Name	Ovadine	Chlorine Bleach (12%)	Virkon/Peroxigard (7%)	Roccal, benzalkonium chloride
Bactericidal	+++	++	+++/+++	+++
Virucidal	++	+++	+++/+++	_
Lipophilic virucidal	+++	+++	+++/+++	+
Sporicidal	+++	+++	++/++	_
Fungicidal	+++	+	++/++	++
Effectiveness in presence of organic matter	+	+	+++/++	++
Effectiveness in presence of soap	++	++	+++/+++	+
Mechanism of action	Oxidizes proteins and interferes with metabolic reactions	Free hypochlorous acid attacks sulfur bonds and general structure in microbial enzymes, other proteins	O2 release alters protein structure	Cationic detergents act against cell wall lipids in bacteria
Neutralize with	Sodium thiosulfate, dilution	Sodium thiosulfate (0.5 thio : 1 hypochlorite), dilution	Dilution	Dilution
Usage notes for working solutions (not accurate for concentrate)	Safe for skin contact, mild odor, color fades as potency declines, not effective if prepared with seawater	Corrosive to skin and metal, strong odor, high risk to fish, volatile, not effective if prepared with seawater	Safe for skin contact, mild odor/few volatiles, color fades as potency declines (Virkon), marked drop in efficacy if prepared with seawater	Safe for short or occasional skin contact, odorless, some risk to fish, not effective if prepared with seawater

Virkon® Aquatic - Directions for General Use

A 1% Virkon® Aquatic solution is recommended for the cleaning and disinfection of surfaces associated with aquaculture including: vehicles, boats, nets, boots, waders, dive suits & other equipment.

Mix the Virkon® Aquatic powder with clean water according to the dilution instructions in the following table.

For heavily soiled surfaces, it is recommended to clean with an appropriate detergent prior to disinfection.

Dilution Instructions

	% Dilution Required		
	0.5% (1:200)	**1.0% (1:100)**	**2.0% (1:50)**
Final Disinfectant Solution Required	**Quantity of Virkon® Aquatic Powder Required**		
1 liter	**5 grams**	**10 grams**	**20 grams**
5 liters	**25 grams**	**50 grams**	**100 grams**
10 liters	**50 grams**	**100 grams**	**200 grams**
25 liters	**125 grams**	**250 grams**	**500 grams**

1. Do not apply Virkon® Aquatic powder directly on surfaces you are trying to disinfect, always mix with water first.

2. Always make your solution in a clean container of known volume.

3. Measure the correct amount of Virkon® Aquatic powder using the calibrated measuring cup provided.

4. Stir the mixture to dissolve the Virkon® Aquatic powder.

5. Apply the solution to the surfaces to be disinfected, wait for the recommended contact time, and follow with a clean water rinse.

One litre of solution is sufficient to disinfect approximately 4 sq meters.

6. Virkon® Aquatic solutions are stable for up to 7 days. Test strips are available to determine the mixed solution's strength.

Version history and notes

Version	Date	Authors	Notes
1.0	2014-05-10	Gord Nash	Initial writing based on references, mainly AQUAVETPLAN.

1.1	2014-06-27	Edits by Stephen Hill	Mostly formatting into final form. Some commitments to suggested practices. CFIA first submission. **CFIA approved 2017.**
2.0	2018-05-03	Stephen Hill	Minor edits. Pre 2018 CFIA resubmission.
3.0	2019-03-21	Stephen Hill	Clarification on various points, including use of freshwater for cleaning.

Memorial University of Newfoundland
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Use of biological safety cabinets (BSCs)

Version	Date of last revision
3.0	2019-03-21

Description

Biological safety cabinets are the principal primary containment device used in the CDRF when working with infectious substances. The directional airflow, HEPA filtration and enclosure protect the operator from exposure to aerosols. Use BSCs when working with infectious material and there is a risk of generating aerosols (opening tubes, using an inoculating loop, pipetting, mixing and homogenizing, needles and syringes, pouring infectious material, etc.) or that involve large volumes of infectious material. Additionally, the BSCs protect samples and materials in the BSC from exposure to operator and environmental contamination. The BSCs in use at the CDRF are **class II type A2** cabinets that use a combination of inward airflow at the front of the cabinet to prevent release of aerosols and downward HEPA filtered airflow to protect the samples. HEPA filtered air is then exhausted through the top plenum and out of the building (fig. 1-2).

Two BSCs are located in room CD-1022. They should remain empty when not in use.

Information on spills within the BSC can be found in the emergency response SOP *CDRF.B.8010.*

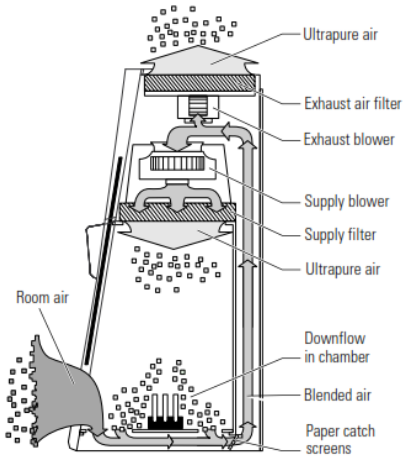


Figure 1-2. Airflow Filtering System

Glossary

- **BSC: Biological safety cabinet**
- **BSO: Biosafety officer**
- **CZ: Containment zone**
- **PPE: Personal protective equipment**
- **RA: Research assistant**
- **SOP: Standard operating procedure**

Safety and responsibilities.

- **The facility manager is responsible for arranging the annual certification through technical services in accordance with NSF 49.**
- **All users of the biosafety cabinet must be trained in person on their use.**
- **The containment RA must check the BSCs daily for correctly indicated airflow and user operation.**
- **Do not use flames in the BSC. Use disposable loops or a bacti-cinerator.**
- **Do not use the BSC as a fume hood. Noxious or flammable chemicals may become concentrated in the hood and corrosive substances damage the HEPA filter and surfaces. Use only small volumes of such substances.**
- **Gloves must be used in the hood and removed when exiting the hood.**
- **Remove watches and jewelry.**
- **Keep the interior of the hood uncluttered to allow proper air movement. Do not block grills of any ventilation areas.**
- **Do not use equipment in the hood that causes air turbulence such as centrifuges or blowers.**

Preparations

1. **Turn off the UV light, if it is on.**
2. **Raise the sash to the height of the top dimple. Wait until the steady airflow light turns green.**
   The green sash position light will indicate (fig. 1-5). An alarm will sound if the sash is raised above this height when airflow is on. If the blower is not on, first press and hold the ON button to activate. Turn on the white light. If the Smartflow performance lights are not green, notify the containment RA (maintenance is required).
3. **Disinfect the interior surfaces with a disinfectant appropriate to the materials being used.**
   See protocol CDRF.B.4010 for guidance on disinfectants. In most cases 70% ethanol is adequate. If bleach or another corrosive is used, rinse twice with sterile water or ethanol afterwards.

4. **Place all materials that will be used for the tasks to be performed in the BSC inside the BSC.**
   It is important to not repeatedly cross the air barrier and disrupt its flow. Think in advance what you will need to avoid repeat trips. Place items towards the back as much as possible. The sash may be temporarily raised higher for loading if needed and then returned to the safe working height.
5. **Confirm that there is inward directional airflow by holding a tissue at the edge of the sash to see if it is drawn in.**

Figure 1-5. Work Position

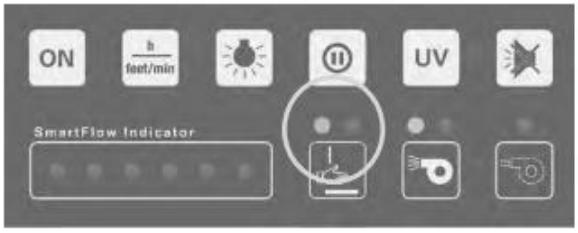


Figure 1-6. Lighted Green LED

Figure 1-7. Fully Closed Position (UV, if applicable)

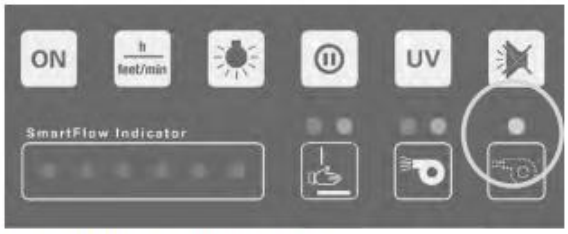


Figure 1-8. Lighted Blue LED

Operating Protocol

1. **Place gloved hands in the BSC and pause to allow the air curtain to stabilize.**
2. **Work with samples such that your operations pass from clean to dirty, left to right.**
   Avoid passing exposed skin over the samples. For sensitive / high risk samples, tape lab coat cuffs to gloves.
3. **When finished, close all containers and pause 5 min to allow time for air to pass through filters.**
4. **Surface decontaminate items. Wipe down interior surfaces including glass.**

With appropriate reagent, usually 70% ethanol or bleach.

5. **Discard gloves in BSC waste and put on a new pair.**
   …or remove outer gloves if wearing two pairs. Alternatively rise gloves with a spray of 70% ethanol.
6. **Remove items. Close the sash to the lower dimple as in fig 1-7. The blue reduced mode indicator will light.**
   Do not move past the dimple. It is not necessary to turn off the fan. For work breaks or pauses, the sash may also be lowered.
7. **Optional: Close the sash all the way and decontaminate with the UV light. This CAN NOT be used as the sole decontamination method.**
   Note that UV decontamination does not replace chemical decontamination. It is useful to degrade DNA for sensitive applications. UV light is harmful to skin and eyes – do not operate with the sash open.

See SOP CDRF.B.8010 for spills in the BSC.

References

Figures from Thermo 1300 series A2 manual.

1. *Laboratory biosafety manual*. (World Health Organization, 2004).

2. *Containment Standards for Facilities Handling Aquatic Animal Pathogens*. (Canadian Food Inspection Agency, Biohazard Containment and Safety Science Branch, 2010).

3. *Canadian Biosafety Standards and Guidelines*. (Government of Canada, 2015).

Version history and notes

Version	Date	Authors	Notes
1.0	2014-02-14	Stephen Hill	First writing, based on Canadian Biosafety Standards and Guidelines, 1st ed. BSC manual and other guides[1–3].
1.1	2015-03-12	Stephen Hill	Edits from Rod Hobbs. **CFIA approved 2017.**
2.0	2018-05-03	Stephen Hill	Review before 2018 resubmission.
3.0	2019-03-21	Stephen Hill	**Approved in 2018.** Review, minor edits.

Memorial University of Newfoundland
Department of Ocean Sciences
Cold-Ocean Deep-Sea Research Facility

CDRF.B.5020

Biosafety procedures for lab equipment

Version	Date of last revision
3.0	2019-03-22

Description

The CDRF labs are equipped with a variety of common pieces of equipment typically found in biology labs. This includes centrifuges, pipettes, incubators, water baths, fridges and freezers, etc. For each of these pieces of equipment, you may refer to the operator's manuals for instructions on their use. Additionally, special infection control precautions must be taken. The forces produced by these instruments may spray liquids or generate aerosols containing infectious material. The following instructions explain how to avoid these releases with some of the key pieces of equipment found in the lab.

Note: Infectious material may not be taken out of the biocontainment zone to be used on equipment outside of the zone. Infectious biologicals must first be inactivated, for instance through fixation or sterilization, before their removal. As an example, frozen samples of infectious material cannot be removed for cryosectioning on the cryostat, since cryopreservation does not inactivate pathogens. In such cases, contact the CDRF manager to discuss relocating equipment into the containment zone, fixing samples prior to relocation or transporting the samples to an appropriate lab space.

Lab equipment can be complicated to operate. Always seek the assistance of trained personnel if you are unsure of correct operating procedures.

Glossary

- **BSO: Biosafety officer**
- **BSC: Biological safety cabinet**
- **CZ: Containment zone**
- **PPE: Personal protective equipment**
- **RA: Research assistant**
- **SOP: Standard operating procedure**

Safety and responsibilities.

- **All users of the facility must be trained on the equipment they will be using.**
- **Do not use equipment you have not been trained on.**
- **The CDRF manager, with the assistance of the CDRF RAs, are responsible for maintaining records on the maintenance of equipment.**
- **Do not relocate equipment without the permission of CDRF staff.**

- **Clean and decontaminate all work surfaces before and after use. Leave the work area uncluttered and ready for the next user.**

Protocols

Centrifuges

Pressures inside the centrifuge may generate aerosols. Breakage of tubes is a further risk. Follow these rules when working with infectious material in the centrifuge.

1. **Confirm that the tubes you are using can withstand the speed of the centrifuge.**
2. **Centrifuge infectious material in sealed tubes with the lid on the rotor or cups.**
3. **Open tubes in the BSC after centrifugation.**
   Do not put centrifuges in BSCs, as they disrupt airflow.
4. **Always properly balance the centrifuge.**
5. **In the event of a spill in the centrifuge, seek the advice of the containment RA and refer to the emergency response SOP *CDRF.8010*.**

Vortexers, shaking incubators and other mixers

Follow these procedures to reduce the risk of spills and aerosol generation.

1. **Where possible, use mixing devices in a BSC.**
2. **Allow time for contents to settle within tubes before opening.**
3. **When vortexing, slowly adjust the speed to keep the fluid from contacting the lids of larger tubes. Use tubes that fully close.**

Bunsen burners and microincinerators

Bunsen burners or microincinerators should be used to sterilize loops, forceps and other metal tools used to handle infectious material. Additionally, open flames may be used to fix slides and for other purposes. These practices can generate aerosols.

1. **The following is the order of preference for using loops: 1. Disposable loops, 2. Microincinerator, 3. Bunsen burner.**
2. **1 or 2 may be, and generally should be, used in a BSC. Open flames cannot be used in the BSC.**

Pipetting

Correct pipetting technique is critical for all aspects of experimentation, but the following instructions should be paid particular attention. Forceful pipetting and mixing may generate aerosols or contaminate the interior of the pipette.

1. **Use plastic, disposable serological pipettes rather than glass when possible.**
2. **Use filter tips for both serological and micropipettors when handling infectious materials.**

3. **Pipette in the BSC.**
4. **Do not oral pipette. Be cautious not to draw fluid into pipette aids.**
5. **Disinfect the exterior of pipettors after use.**
6. **Always return micropipettors to their holders after use.**

Needles

The use of needles should be limited as much as possible to avoid needle stick injuries and injection exposure to the operator.

1. **Use the appropriate needle to the task. Do not alter the needle.**
   Do not bend or cut the needle.
2. **Place used needles in biohazard sharps container. Do not recap.**
3. **Full sharps containers to be autoclaved before disposal.**
4. **Collect sharps containers for incineration as with anatomical waste.**
   See SOP CDRF.B.6010.

Vacuum pumps and aspirators

Within the CDRF vacuum pumps or tap aspirators are used for filtration methods. If potentially infectious material is being filtered or aspirated with a vacuum, there is a risk of generating aerosols or contaminating the workings of the pump or lines.

1. **Use an inline, hydrophopic filter trap between the liquid end and the suction end.**
2. **Use a flask trap to prevent fluid from entering the pump.**
   Empty this flask whenever it is half-full into a container for autoclaving. Alternatively, fill the flask trap with disinfectant sufficient to kill the biohazard agent.

Chemical fume hoods

Chemical fume hoods within the CDRF are not to be used in place of the BSCs or vice versa. Fume hoods do not adequately protect the user from infectious substances or the sample from contamination. BSCs partially recirculate air and may be damaged by substances that should be used in a fume hood.

Good Microbiological Practices

Procedures to Minimize Aerosol Hazards

Opening Tubes

- Manipulate infectious materials within a biological safety cabinet.
- Upon opening, unscrew the cap slightly and wait a few seconds before removing it.

Inoculating Loop

- Use a micro-incinerator or a disposable loop instead of a bunsen burner.
- Allow the inoculating loop to cool before any procedures.

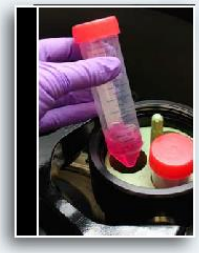
Syringes/Needles

- Withdraw needles from bottles using disinfectant-soaked absorbent pads wrapped around the bottle cap.
- Use locking syringes.

Pipetting

- Use "to deliver" pipettes calibrated to retain the last drop.
- Use pipettes with plugs.
- Discharge pipettes close to the fluid level and let the contents run down the wall of the container.
- Never forcefully expel infectious materials from the pipette.

Centrifugation



- Centrifuge infectious material in closed containers, placed in sealed safety cups or rotors.
- Open cups in a biological safety cabinet.
- Maintain the centrifuge to ensure that it is clean and the gaskets and O-rings are not compromised.
- Wait 5 minutes before opening the centrifuge after each run to allow any aerosols to settle.

Breakage

- Avoid the use of glassware where possible.
- Use plastic tubes, flasks and bottles.
- Use screw-capped tubes and bottles rather than plugs or snap caps.

Mixing and Homogenizing

- Ensure the lab blender has a gasket lid and leak proof bearings.
- Wait a few seconds before opening a lid after mixing.
- Use a vortex, instead of inverting the cultures.

Pouring Infectious Materials

- Perform your work over plastic-backed absorbent material.
- Wipe the rim of the tube with disinfectant-soaked absorbent paper to remove potential contamination on the outside of the tube.

References

1. *Laboratory Biosafety Manual*. (World Health Organization, 2004).

2. *Containment Standards for Facilities Handling Aquatic Animal Pathogens*. (Canadian Food Inspection Agency, Biohazard Containment and Safety Science Branch, 2010).

3. *Canadian Biosafety Standards and Guidelines*. (Government of Canada, 2015).

Version history and notes

Version	Date	Authors	Notes
1.0	2014-02-25	Stephen Hill	Initial writing, based largely on CBSG and other guides[1–3]. CFIA first submission.
1.1	2015-03-17	Stephen Hill	Addition of aspirator to pump SOP. **CFIA approved 2017.**
2.0	2018-05-04	Stephen Hill	Minor edits. Pre 2018 CFIA resubmission.
3.0	2019-03-22	Stephen Hill	Approved in 2018. Minor edits, note to incinerate sharps.

Memorial University of Newfoundland
Department of Ocean Sciences
Cold-Ocean Deep-Sea Research Facility

Sterilization, biohazardous waste and the autoclave

Version	Date of last revision
5.1	2020-11-03

Description

The university biosafety SOPs on the Management of Biohazardous Waste (BSOP-01) may be found on the website of MUN Biosafety. This SOP serves as a CDRF specific reference.

https://www.mun.ca/health_safety/OHSMS/LSMS/BSMS/Biosafetyoperatingprocedures.php

Sterilization is a process which completely kills or inactivates infectious organisms. At the CDRF, autoclaving is the principal method of sterilization. All waste leaving the CZ will either be autoclaved or incinerated. CDRF autoclaves are Getinge pass-through autoclaves; Materials are placed in the autoclave within an area of higher infectious risk, sterilized, and then passed to an area of lower risk or the exterior of the CZ.

The following protocols describe the usage of the floor model Getinge model 544LS autoclaves. Autoclaves achieve sterilization through high temperatures and pressure. Proper use of the autoclave will ensure effective sterilization. It is important to have a thorough knowledge of correct autoclave use to prevent the release of infectious material from the CZ to other labs and the environment. The autoclave will also be used to sterilize equipment and reagents used in experiments. This prevents contamination of sterile materials and cross-contamination from non-sterile materials.

In addition to the autoclaves, some waste will be incinerated. This includes all anatomical waste and carcasses as well as sharps.

Glossary

- **BI: Biological indicator**
- **BSO: Biosafety officer**
- **CZ: Containment zone**
- **EHS: Environmental Health and Safety (health.safety@mun.ca)**
- **PPE: Personal protective equipment**
- **RA: Research assistant**
- **SOP: Standard operating procedure**
- **WWTP: Wastewater treatment plant**

Safety and responsibilities.

- **Memorial University's EHS will ensure that autoclaves are receiving a biennial inspection by individuals qualified under the *Boilers, Pressure Vessels and Compressed Gas Regulations.***
- **Anyone using the autoclave must receive hands-on training from the containment RA before use.**
- **The containment RA is responsible for collection and sterilization of common waste, ensuring that waste is properly labelled and routine monitoring of autoclave efficacy.**
- **The facility manager is responsible for maintaining records of autoclave validation, use and maintenance**
- **Wear all suitable PPE, including safety glasses, hot mitts and lab coat when unloading the autoclave.**
- **Wear lab gloves under heat insulated mitts to protect from hot moisture soaking through mitts.**
- **Each week, the containment RA will check and clean the drain trap.**
- **Vessels must be vented to allow pressure to escape. Partially unthread bottle caps and do not seal bags.**
- **Be sure that plastics are autoclave safe.**
- **Do not overload the autoclave.**
- **Use autoclave gloves to remove hot contents after autoclaving. The interior and gasket will be hot after a run.**
- **Do not autoclave items that will generate toxic or corrosive vapours when heated, including radioactive materials.**

Preparations

Determine what cycle conditions you need for your material. Label all items to be autoclaved with contents and indicator tape. Consult with CDRF staff for assistance. Determine if your waste is to be autoclaved or incinerated (see table below).

Table 1: Waste treatment options for biohazardous waste.

Biohazard type	Decontamination by steam autoclave prior to landfill disposal	Alternative treatment method (required if "no" listed in previous column)
Animal anatomical waste	No	Incineration
Animal blood/body fluids	Yes	
Human anatomical waste	No	Incineration
Human blood/body fluids	Yes	
Microbiology Lab Waste: Primary animal cell lines Established animal cell lines Primary human cell lines Established human cell lines Recombinant DNA/RNA Bacteria Viruses	Yes	
Fungi	Yes	
Venoms/Toxins	Yes	
Parasites	Yes	
Plants, plant parts and plant pests	No	Incineration
Plant cells and cell lines	Yes	
Prions	No	Incineration
Sharps (needles, blades, etc.)	No*	Incineration

Data compiled from Table 3: Summary of treatment options for biomedical waste and Table 4: Summary of disposal options for untreated biomedical waste, Guidelines for the Management of Biomedical Waste in Canada.

* Sharps containers must be autoclaved prior to pick up for incineration.

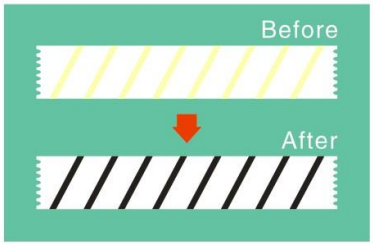
Protocols

Collection of solid waste

1. **Place all solid infectious material and live cultures for disposal in biohazard bins lined with an autoclavable biohazard bag.**
   Biohazard waste bins are found throughout the CDRF. The bag should be clear without any biohazard symbol markings. Liquid biohazard waste should be autoclaved in flasks. Anatomical waste, including carcasses should be put in incineration containers (see below).
2. **When the bag is half full, or at the end of the week, remove the bag and close *loosely* with a twist tie.**
   There should be enough of a gap in the neck to allow steam to enter.
3. **Place a piece of autoclave tape on a card or item within the bag as well as on the outside of the bag.**
   You may attach a string to the indicator to help locate after the run.
4. **Attach biohazardous waste tag.**

This should indicate the project and PI name, date and description of waste.

5. **Follow instructions below to autoclave waste on WASTE cycle.**
6. **Recover waste from the other side of the pass-through autoclave.**
7. **Ensure that indicator strips are positive.**
   Positive autoclave tape indicator is brown stripes on the white tape.



8. **Remove the hazardous waste tag, place bags in unmarked black garbage bags and deposit waste in storage location until biological indicator results for the week are done.**
9. **Provided BI result is okay, dispose stored waste in the dumpster.**

Sharps disposal

1. **All sharps should be disposed of in approved sharps containers.**
   The containers should be labelled with a biohazard symbol or tape.
2. **Close the lid of the sharp container and set aside for incineration.**
   See below on anatomical waste for incineration instructions.

Preparation of materials for the autoclave

In addition to waste, the autoclave may be used to sterilize small equipment, such as surgical tools and pipette tips, non-volatile liquids and glassware. The following is a sample list of items which may or may not be autoclaved

Autoclave compatible	*Incompatible*
Tissue culture flasks	Heat labile materials
Surgical Instruments	Acids, bases, organic solvents
Glassware	Chlorides, sulfates
Pipette tips	Seawater
Media solutions	Chlorine, hypo-chlorites, bleach
Animal food and bedding	Non-stainless steel
Waste	Polystyrene
Polypropylene	Some types of polyethylene, high density
Stainless steel	polyethylene
http://www.ehs.ucsb.edu/units/labsfty/labrsc/factsheets/Autoclaves.pdf	

1. **Verify that solid items are autoclave safe and wrap in autoclave paper or place in a loosely opened autoclave bag.**

2. **Liquids should fill not more than 2/3 of the total vessel volume and must be vented.**
   Vent by using a lose covering, such as aluminum foil or partially unthreading bottle caps. Air must be able to enter the vessel to avoid rupturing the container and damaging the autoclave as well as for effective sterilization.
3. **Label each item with a piece of autoclave tape.**
4. **Place waste, solids or liquids in an autoclave bin.**
   Do not use any other sort of bin, such as Tupperware, which will melt.

Using the autoclave

1. **If the screen is off, press the green ON button to turn on the controls.**
   If you are starting a cold autoclave, it will take approximately 30 min for the boiler to heat up before use.
2. **Open the door.**
   This may be done by pressing the OPEN DOOR switch and gently pushing down on the door handle. The door cannot physically be opened when a run is in progress due to the gasket seal. Additionally, the door cannot be opened on the clean side if the autoclave has not cycled (i.e. the interior of the autoclave is not sterile). Stand back from the door opening after a run as some steam may at first be released.
3. **Load the autoclave.**
   Do not place items on floor of the autoclave.
4. **If autoclaving volumes of liquid (waste or media) or sludge greater than 1 L, position the load probe in the middle of the largest volume. Place a steam indicator in the interior of bagged material.**
   The load probe will ensure that large volumes of liquid reach sterilization temperature. Only the autoclave in room CD-1027 (off of effluent treatment) has a load probe. The probe is delicate – be mindful to remove it gently after the run is complete and return it to the holster.

FIGURE 4–6. LOAD PROBE PLACEMENT

Place the load probe into the center of the largest container with the greatest volume of liquid and locate it in the coolest part of the chamber (usually near the chamber drain).

5. **Close the door.**
6. **Select cycle, press ENTER and then press OK.**
   Use the console up and down arrows to scroll and press ENTER to preview the cycle. If you do not press ENTER before pressing OK, the previously run cycle will start, not the highlighted one. See below for current cycle information. If you require different cycle conditions, contact the CZ RA.
7. **Press START. Fill out the autoclave use sheet.**
   The cycle will start. If you need to abort the cycle, press ABORT and allow the autoclave to vent.
8. **Confirm that the cycle is complete and that all indicators are positive.**
   The printer tape and screen will indicate CYCLE COMPLETE. The PROCESS COMPLETE indicator will be green. If it is red, the PROCESS FAILURE light is red, the printout indicates CYCLE ABORT or there are any other failure messages, your items are not sterile. Contact the facility manager or containment RA for assistance.
9. **If any of the above indicators are negative, the material cannot be considered sterile.**
   Record this in the log book and attempt resterilization with new indicators.
10. **Remove contents from the opposite door if they are leaving the CZ.**
    Take all precautions listed above. Moisture from condensation on exposed surfaces is hot enough to burn exposed skin.

Autoclave cycles

The following cycles are currently programmed. Contact the facility manager or containment RA if you require alternative operating conditions. Gravity cycles are designed for solids and vent rapidly with a drying stage. Liquid cycles vent slowly to prevent fluids from boiling over during a rapid pressure release. Prevac cycles draw a vacuum to remove air, allowing enhanced steam penetration and shorter run times. In the case of large loads, the following cycle times may be insufficient to achieve sterilization. Different loads should be tested with biological indicators as described below. The load probe may also be used, especially on liquid runs, to be certain temperature has been reached.

Number	Program	Program type	Load probe	Sterilize time	Sterilize temp °C	Dry time
1	DRY Waste	Prevac	No	0:30:00	121.1	0:00:15
2	Glassware	Prevac	No	0:10:00	132	0:10:00
3	Instruments	Prevac	No	0:30:00	121.1	0:30:00
4	Flash	Prevac	No	0:04:00	132	0:10:00
5	WET waste	Gravity	No	0:30:00	121.1	0:00:15
6	Liquid < 1 L	Liquid	No	0:30:00	121.1	0:00:00
7	Liquid > 1 L	Liquid	No	1:00:00	121.1	0:00:00
8	Liquid < 1 L	Liquid	No	0:15:00	121.1	0:00:00
9	Sludge	Effluent	Yes	1:00:00	121.1	0:00:00

Validation of sterilization and maintenance

The containment RA is responsible for regular maintenance and monitoring of autoclave efficacy with biological indicators. All users should use indicator tape, on each load.

Biological indicator testing to be done every week on a waste or sludge load for each autoclave, or when a new load type is first introduced. If a waste load is not being run each week, run the BI on the next waste or sludge load being decontaminated. All waste must be held until the BI test is complete for that week.

1. **Place a *Geobacillus stearothermophilus* biological indicator inside the waste bag or test material. Place in the centre of the load in the centre of the autoclave.**
   This should be the location where it will be the most difficult for heat and steam to reach. You may attach string to the indicator to help locate after the run.
2. **Take another tube from the same lot and set aside.**
3. **Run the cycle.**
4. **Upon completion of the run and cooling, remove the indicator from the test bundle and incubate it following the directions of the manufacturer.**
   For the NAMSA biological indicators, this is at least 24 hours at 55-60 °C. Test immediately after incubation.
5. **Activate both indicators by crushing.**
6. **Incubate at 56°C for 24 hours and observe for colour change. Incubate the second, un-autoclaved packet at the same time to verify that the indicator is viable.**
7. **A change in colour to purple indicates a pass (i.e. sterility achieved) while colour change to yellow with turbidity indicates failure.**
   If the second, negative control turns yellow, the indicator lot should be replaced.
8. **Record all results in the biological indicator test log for each autoclave.**
9. **Upon a failure the load cannot be considered sterilized.**

Anatomical waste and alternatives to autoclaving

Animal carcasses, anatomical waste and sharps may not be decontaminated by autoclaving. This kind of waste is collected for off-site disposal. Please contact the facility manager or the Institutional BSO for more information. For toxic fluids, consider sterilization by filtration.

Anatomical waste may be either incinerated or collected for specialized collection and landfill disposal. Since there is no incinerator available for fish waste in Newfoundland, the latter is preferred for large amounts of waste, such as fish carcasses.

Collection of anatomical waste

1. **Use a separate waste bin for collecting anatomical waste and supplies that are grossly contaminated with blood or tissues (e.g. paper towels).**
   Line with an autoclave bag.
2. **When half full, or complete for the day, close bag and attach a hazardous waste materials tag indicating the project PI, date and type of waste.**
3. **If incinerating, place this bag in an orange incineration bin. If sending to landfill, place in an orange bag labelled "INTERNATIONAL WASTE."**

Do not overfill bags such that they are too heavy or difficult to close.

4. **Place the bag in the mort freezer in room CD-1026.**
   Do not use any other freezer for anatomical waste.

Incineration

1. **The incineration container must undergo secondary disinfection as per SOP CDRF.B.4010 (spray with ethanol or bleach) before exiting the CZ.**
2. **Complete a hazardous waste disposal form to arrange pickup of the bins or for delivery of new bins.**
   The containment RA will do this. Most incineration is done by Stericycle, phone: 506.855.4404 (site 003, account 7025836). Stericycle provides 28L bins for disposal.

Landfill disposal

Local CFIA can approve disposal of anatomical waste as "International waste.[6]" Waste disposed of in this manner is collected by a certified hauler who will provide an appropriate container and notify the CFIA of the routing and destination of the waste disposal. At the landfill site, a pit will be dug for this waste where it will be deposited and buried. As of 2020-09-23, the Canadian Food Inspection Agency – Office of Biohazard Containment and Safety (CFIA-OBCS), CFIA National Aquatic Animal Health Program (CFIA-NAAHP) and local CFIA veterinarians approve the use of the Government of Canada's International Waste Directive (TAHD-DSAT-IE-2002-17-6, effective 2012-10-15) disposal procedures for the current (effective 2020-05-15) identified facility aquatic pathogens and aquatic species.

1. **Waste should at this point be double bagged, in one autoclave bag with an outer orange bag labelled INTERNATIONAL WASTE and undergo secondary disinfection.**
2. **Contact the waste hauler, who will arrange the delivery of an IW waste bin.**
   This is an approximately 6' cube sized bin. It is best to wait until a full freezer load is prepared for disposal to maximize its use. Instruct the workshop and driver to leave this bin near the others in front of JBARB. Approved hauler contact info: GFL Environmental (67 Major's Path, St. John's, NL A1A 4Z9) – Shannon Crawley (he), 728-2109. Waste will be transported to the approved landfill, Robinhood Bay (340 E White Hills Rd, St. John's, NL A1A 5J7).
3. **When you are ready for pick-up, notify the hauler to arrange the pick-up.**
   They will need 2-3 days to notify the landfill and CFIA.
4. **Load the bin with as short a time as possible between loading and pick-up, e.g. morning of.**
5. **Zip tie the bin closed and label, "INTERNATIONAL WASTE, not for general waste disposal.**
   The bin is now ready for pick-up.

Record keeping

1. **All users should fill out the usage log next to each autoclave as shown on sheet.**
   Records will be maintained by the CDRF Manger.
2. **All users should indicate the result of negative indicators or equipment failures in the autoclave binder.**

3. **The containment RA should record the results of biological indicator tests and repairs or maintenance in the binder.**

Maintenance

Maintenance should be conducted as required and by the following schedule. Perform maintenance in the morning on a cool autoclave. The operator's manual should be consulted for details on conducting maintenance.

Daily maintenance (each morning upon first use)

- **Review cycle printouts for errors on previous runs.**
- **Clean the sediment screens (see operation manual for instructions).**
- **The autoclaves are programmed to automatically flush the steam generators.**

Weekly (each Monday morning)

- **Clean interior and exterior as needed.**
- **Wipe down door gaskets.**

Annually

- **Preventative maintenance inspection by authorized service technician.**

Materials

Name	Vendor	Cat No.
Self-Contained Biological Indicators (SCBI), NAMSA	VWR	95029-702

- Autoclave bags and ties
- Autoclave bins
- Autoclave tape
- Hot mitts

References

1. *Laboratory biosafety manual*. (World Health Organization, 2004).

2. *Containment Standards for Facilities Handling Aquatic Animal Pathogens*. (Canadian Food Inspection Agency, Biohazard Containment and Safety Science Branch, 2010).

3. *Canadian Biosafety Standards*. (Government of Canada, 2015).

4. Getinge 400/500LS Series Steam Sterilizer User Manual.

5. St. Andrew Biological Station SOP SABS-WET-1135 (8 Feb 2013).

6. International Waste Directive. https://www.inspection.gc.ca/animal-health/terrestrial-animals/imports/import-policies/general/2002-17/eng/1321050654899/1323826743862#legis, Retrieved 2020-05-10.

Version history and notes

Version	Date	Authors	Notes
1.0	2014-03-19	Stephen Hill	First version, based on biosafety manuals[1–3].
2.0	2014-06-17	Stephen Hill	Edits from KG. Removal of cart, which can't be used because of flood bariers. Addition of Ver 1 cycle protocols.
2.1	2014-06-24	Stephen Hill	Removal of use of F0 in favour of load probe absolute temperature. Pre-operation. Changes in BI choices. CFIA first submission.
2.2	2014-07-24	Stephen Hill	First review from CFIA. Clarified location of BI. Secondary decon of sludge container.
3.0, 3.1,3.2	2014-09-12	Stephen Hill	Modified from MUN draft biohazard SOPs. 3.1: editing.
3.3	2014-03-17	Stephen Hill	After Rod Hobbs review. Note, waste stored pending BI results.
3.4	2015-05-05	Stephen Hill	Minor edits. Pre-submission. **CFIA approved 2017.**
4.0	2019-03-26	Stephen Hill	Updated Stericycle contact information. Added reference to MUN Biosafety BSOP-01. Removal of some sludge information as this is now being chlorinated. Clarification that sharps to be incinerated. Updated autoclave cycle program information.
5.0	2020-05-10	Stephen Hill	Added new International Waste option for disposal, disposal form info.
5.1	2020-11-03	Stephen Hill	Added landfill info. APPROVED by CFIA.

Filter Backwash and Sludge Treatment

Version	Date of last revision
3.0	2019-03-26

Description

Work in the containment zone (CZ) of the CDRF will generate wastewater containing pathogenic material which could be harmful to the local ecology if released untreated to the surrounding waters. Aquatic containment labs conducting *in vivo* experiments are required to treat all wastewater before releasing it. At the CDRF, all drains within the CZ, except for the washrooms, lead to the wastewater treatment room. The treatment system consists of an incoming effluent line from the containment facility. The flow splits into two streams to enter parallel drum filters (DF5.1 and DF5.2) for particulate removal. An automated float switch activated system triggers backwash of the screens in DF5.1 and DF5.2 for clearing as needed. Backwash water collects in SC5.1. Wastewater from each filter then recombines into a single collection tank (CT5.1) where it is lifted via one of two parallel pumps (P5.1 and P5.2) to one of two parallel AFM media filters (CF5.1 or CF5.2) for further particulate removal. The waste stream then continues from the filter into a single line containing two UV disinfection units (UV5.1 and UV5.2) connected in series. Settled particulate material from SC5.1, along with backwash water from the AFM media filters CF5.1 and CF5.2 (manual process; as required) is collected into two 940L tanks (BWC5.1 and BWC5.2) for chlorine disinfection prior to release.

Glossary

- **BSO: Biosafety officer**
- **CZ: Containment Zone**
- **PPE: Personal Protective Equipment**

Safety and responsibilities.

- **All personnel must first obtain access permission from the BSO and the facility manager as detailed in the biosafety manual introductory material (conditions of access, roles and responsibilities) and receive biosafety training.**
- **Training in SOP CDRF.B.2010 on personal protective equipment and habits.**
- **All users handling chemicals must be aware of WHMIS requirements.**

Protocols

Settled particulate material from SC5.1, along with backwash water from the AFM media filters CF5.1 and CF5.2 (mixed) is collected into two 940L tanks (BWC5.1 (SC5.1 contents) and BWC5.2).

A stock chlorine treatment solution (28.2 g/L HTH Sock It) is added to each of the two BWC tanks as they are being filled to allow maximum mixing and contact time. After 1 hour of chlorine treatment, both tanks are tested for residual chlorine levels by AquaChek dip strips (or YSI Photometer 9500 Chlorine test, if needed).

Solutions

Chlorine solution, concentrated (28.2 g/L @ 65% available chlorine)

1. **Dissolve 28.2 g HTH Sock It Chlorine Shock Treatment (www.poolsuppliescanada.ca, item 14001) in 1 L dH_2O, gently shake to dissolve.**

   Prepare fresh as required and store at room temperature. Calcium hypochlorite (available chlorine content 65%) will not dissolve completely into solution, some insoluble particulate will remain.

10% Sodium Thiosulfate Solution (chlorine neutralizer)

1. **Dissolve 100 g $Na_2S_2O_3$ (Pentair, item ST1A) in dH_2O to a final volume of 1 L in dH_2O, mix by stirring (or shaking) and store at 4°C.**

   Prepare fresh weekly.

Water treatment and testing

1. **Treat contents of BWC5.1 and 5.2 with 1L of chlorine solution, providing ~20 ppm chlorine based on tank volume.**
2. **Add while filling to allow maximum mixing and contact time.**

   Mix by stirring for 5 minutes if added post-filling.

3. **Test chlorine residual (see protocols below).**

   Wait one hour after addition of chlorine before testing.

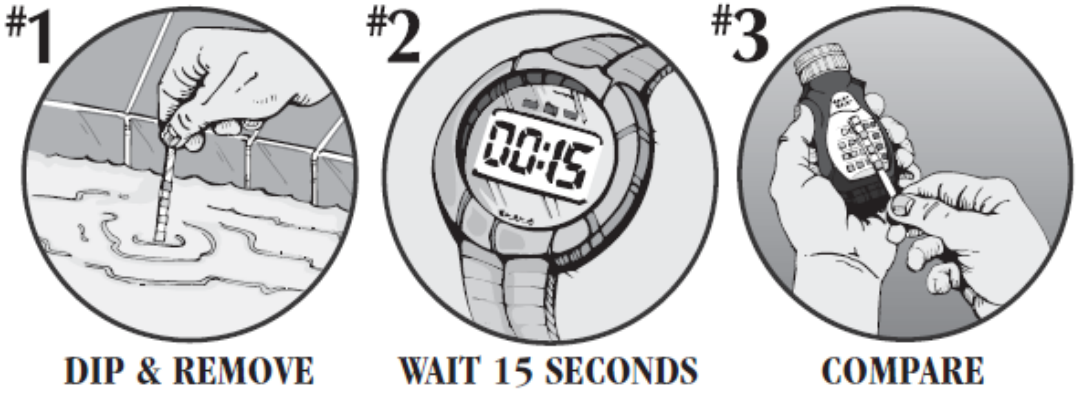
4. **If levels are (as per AquaChek free chlorine strip key):**
   a. **0, 0.5, 1 ppm:** add an additional 1L of stock chlorine treatment, stir and retest after 1 hour.
   b. **3 ppm:** considered disinfected, allowable residual levels for direct discharge.
   c. **5 ppm+:** considered disinfected, neutralize residual chlorine by addition of 10% sodium thiosulfate (Na2S2O3) prior to discharge.

Chlorine testing

Test directly from the tanks using the Dip strip method or collect a 50 ml water sample from each tank if using photometer method for residual chlorine testing. (See method descriptions following).

Dip strip test method

AquaChek®



1. **Dip a strip into water and remove immediately**
2. **Hold strip level for 15 seconds (do not shake excess water from strip)**
3. **Compare pH, Free Chlorine, Total Alkalinity and Stabilizer pads (in that order) to color chart on the label.**

IMPORTANT: *Keep cap closed tight between uses. Store at room temperature. Use by expiration date on cap.*

YSI Photometer (9500) Test Instructions

The YSI Chlorine/Chloramines test uses the DPD method. This method is internationally recognised as the standard method of testing for chlorine and other residuals. In the YSI method the reagents are provided in tablet form for maximum convenience and simplicity of use. Free chlorine reacts with diethyl-p-phenylene diamine (DPD) in buffered solution to produce a pink coloration. The intensity of the color is proportional to the free chlorine concentration.

1. **The photometer is programmed for free chlorine and for the chloramine stages. Use program Phot 71 Free Chlorine then select 'Follow On' from screen options to continue test for program 72 Monochloramine and again for program 73 Dichloramine.**

   Determining only Free Chlorine, DPD No 1, no need for follow on portion of protocol.

2. **Rinse test tube with sample leaving two or three drops of sample in the tube.**
3. **Add one DPD No 1 tablet, crush tablet and then fill test tube with sample to the 10 ml mark. Mix to dissolve tablet.**
4. **Select Phot 71 on photometer.**
5. **Take photometer reading immediately (as result may drift on standing), in usual manner - see photometer instructions. The result represents the free chlorine residual as mg/l Cl_2.**

Materials & equipment:

Treatment and normal testing

- HTH Sock It Chlorine Shock Treatment (454 g) (Pool Supplies Canada)
- AquaChek Yellow 4 in 1 Chlorine Test Strip (Pool Supplies Canada)
- Proline Dechlorinator Sodium Thiosulfate (Pentair)

YSI testing

- YSI DPD No 1 Tablets
- YSI 9300 or 9500 Photometer
- Round Test Tubes, 10 ml glass (PT 595)

Appendix

Product Information and Instructions

HTH ® SOCK IT
FAST ACTION
SHOCK TREATMENT

Kills Bacteria, Controls Algae, Destroys Organic Contaminants,
No Need to Predissolve

ACTIVE INGREDIENT:
CALCIUM HYPOCHLORITE.... 68%
INERT INGREDIENTS:.....32%
TOTAL.....100%
MINIMUM
AVAILABLE CHLORINE...65%

NET WT. 1 LB. (16 OZ.) (0.45 KG)

EPA REG. NO. 1258-913
EPA EST. NO. 1258-TN-1

Version history and notes

Version	Date	Authors	Notes
1.0	2017-10-16	Gord Nash	Original notes 16-10-17
1.1	2018-01-18	Gord Nash	Revisions and formatting 17-01-18
1.2	2018-01-08	Stephen Hill	Review and editing
3.0	2018-03-26	Stephen Hill	Minor edits. Included in 2019 PVTR as follow up to on-site meeting. APPROVED 20-10-09

Emergency ETS Purge

Version	Date of last revision
1.0	2021-04-08

Description

The effluent treatments systems of the CDRF containment zone (CZ) primarily rely on UV disinfection. Water which absorbs UV, either through dark colouration or the presence of UV absorbing chemicals, can cause the UVs to drop below validated threshold levels and consequently shut-down water flow in the CZ. This is primarily the result of the addition of large volumes of tap water, which is coloured brown at the OSC, however other events can have this effect unexpectedly.

When this happens, it is not possible to pass wastewater through the system in a normal manner without exceeding operational thresholds for UVT. In this case, release of wastewater must either be manually controlled, or wastewater pumped out and chemically treated as in CDRF.B.6030.

Glossary

- **BSO: Biosafety officer**
- **CZ: Containment Zone**
- **ETS: Effluent Treatment Systems**
- **FST: Facilities Services Technician**
- **PPE: Personal Protective Equipment**
- **UV: Ultraviolet**
- **UVT: UV Transmission**

Safety and responsibilities.

- **All personnel must first obtain access permission from the BSO and the facility manager as detailed in the biosafety manual introductory material (conditions of access, roles and responsibilities) and receive biosafety training.**
- **Training in SOP CDRF.B.2010 on personal protective equipment and habits.**
- **All users handling chemicals must be aware of WHMIS requirements.**
- **This protocol is only to be performed by the CDRF manager, or properly trained workshop staff, such as the water quality specialist.**
- **Working alone policies should be followed (i.e. by regularly checking in with a contact). Since these events can often happen after normal hours, notify the FST that you are working in the CZ.**

Protocols

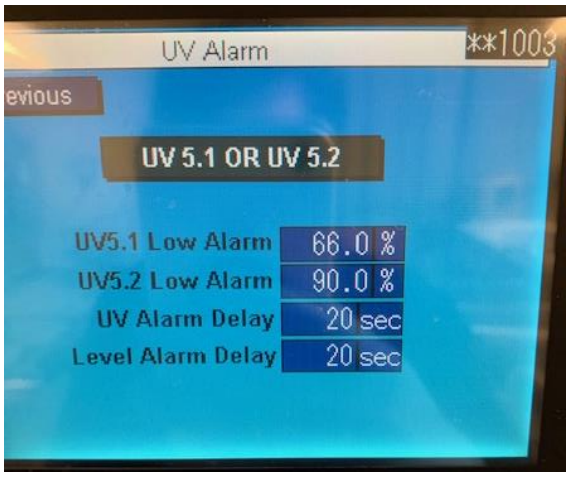
Manual release of wastewater using UV

The normal, automatic operation of the ETS controls the flow of water through the UVs by adjusting the speed of lift pumps P5.1 or P5.2. The UVs have a constant flow of clean cooling seawater going through them to maintain UV temperatures in a low or no-flow event. This cooling water can also serve to dilute dirty water to levels that achieve an adequate UVT reading, however the flow of wastewater must be slowed and no new water can be added to the system.

In this case, the water supply auto-valves in room CD-1010 will be in the off position, the lift pumps off and the system in alarm due to low UVT. As cooling water slowly purges the UVs (while they remain on), UVT will return to above the threshold level, consequently adding new, dirty wastewater to the UV chambers and shutting the system down again. Although it is possible for the system to very slowly clear water over time in this intermittent manner, it should not be allowed to happen as this can result in the auto-valve batteries wearing down (they are powered from the batteries and only recharge from the main power). In such a situation it is possible for the valves to remain open even when in an alarm state, potentially flooding the pit. **ETS alarms should not be ignored for this reason**. The following steps may be taken to release wastewater in a controlled manner which doesn't constantly cycle the auto-valves.

1. **Force the CD-1010 auto-valves into a closed position by temporarily raising the UVT threshold settings.**

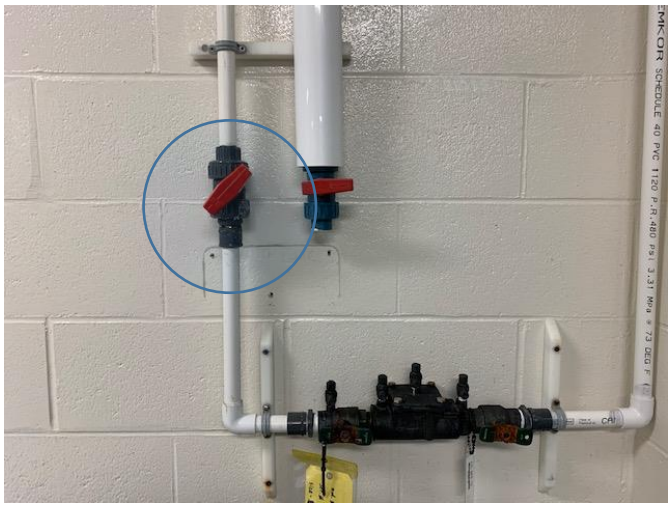
   This can be accessed from the UV Alarm menu. Change the thresholds to 100%.



2. **On the pump panels, turn both pumps off.**

3. Optional: Increase the flow of the cooling water.

The left handle on the door-side wall by the autoclave. You must be conscientious of header volume if you do this. Confirm availability of water with the workshop before increasing flow.



4. Turn off the drum-filter backwash jets by turning to HAND.

5. **Adjust the pump speed of one of the pumps to 0 Hz and switch this pump to HAND ON.**
6. **Once the cooling water has returned one of the UVs to the correct threshold, begin slowly turning up the pump speed of the pump in manual mode (HAND ON).**

   Go in steps of 2 Hz. Note that the pumps will not actually begin lifting water out of the pit until around 15Hz, however you may begin to see a decrease in UVT as water is pushed out of the media filters. If very low speeds still result in high UVT values, consider chemical disinfection as below.

7. **So long as the UVT remains above threshold, continue to increase the speed. If UVT drops below threshold, immediately turn the pump off and return to step 6.**
8. **Purge all water in this manner until the collection tank depth drops to the level of the intake washouts, ~ 30 cm.**

   Do not allow the water to drop lower than this at any time. You may need to turn off the pumps.

9. **Allow water to return to the system by resetting the UV parameters from step 1.**
10. **As seawater further dilutes the remaining dirty water, you will be able to increase the pump speed to the prior value.**

    See the log book for normal pump speeds. If the system returns to an alarm state, return to step 1.

11. **Once a normal pump speed has been reached, return both pumps to automatic mode, turn the drum-filter backwash jets on and verify that alarm settings are correct.**
12. **Monitor water levels in the CT during this process. If water gets too high because you are unable to sufficiently increase pump speed, return to step 1.**
13. **Continue to monitor alarm states remotely.**

Manual release of wastewater using chemical disinfection

In some events, a slow release of wastewater through the UVs is not possible. This generally happens because the water is so severely fouled that even very low pump speeds will produce high UVT values. In such a case, it may not be possible to clear wastewater in a time that is reasonable for fish health or staffing reasons. The process may be expedited by using the backwash collection tanks as a location to pump out the collection tank.

1. **Follow steps 1-4 of the above UV protocol.**

2. **Attach the outlet of the pneumatic (diaphragm) pump to one of the backwash collectors.**

3. **Place suction hose at bottom of collection tank, be sure to not block inlet.**
4. **Turn on diaphragm pump.**

Approximately two pulses / sec.

5. **Switch valve routing to second tank once first is full.**
6. **You may at this point attempt the above UV protocol.**
7. **At the same time, disinfect the backwash collection tanks as per protocol CDRF.B.6030.**

8. **Once a tank has passed disinfection verification and been neutralized, water can be released to the drain.**
9. **Repeat as needed. The process can also be hastened by disinfecting within the drum filter tanks and the collection tanks.**

Disinfection of this water must be verified as with the collection tanks.

Version history and notes

Version	Date	Authors	Notes
1.0	2021-04-08	Stephen Hill	Original draft.

Memorial University of Newfoundland
Department of Ocean Sciences
Cold-Ocean Deep-Sea Research Facility

CDRF.B.8010

CDRF biocontainment emergency response procedures

Version	Date of last revision
4.0	2018-05-02

Description

Memorial University's Enterprise Risk Management office is responsible for facilitating the development, implementation and maintenance of an Emergency Management Program, which includes all emergency management plans for Memorial's campuses and the coordination of all response activities requiring a Level 2 or Level 3 response. The following material expands upon the St. John's campus ERP to cover items specific to the CDRF, and biocontainment in particular. Emergencies in a biocontainment setting expand on the basic response in that they must consider measures to prevent the accidental release of infectious material and avoid exposure of unprotected individuals. In general, risk to humans from aquatic pathogens is minimal, making the maintenance of containment desirable, but not ultimately necessary in the face of serious accidents. Events that present no direct human risk, but which threaten containment are also described below.

Glossary

- **ERP: Emergency response plan**
- **BSO: Biosafety officer**
- **CZ: Containment zone**
- **LAI: Laboratory Acquired Infection**
- **MUN: Memorial University of Newfoundland**
- **OSC: Ocean Science Centre**
- **PI: Principal Investigator**
- **PPE: Personal protective equipment**
- **RA: Research assistant**
- **SOP: Standard operating procedure**
- **WWTP: Waste water treatment plant**

Safety and responsibilities.

- **Staff should be aware of existing MUN fire and lab safety procedures.**
- **CDRF staff should include people trained in first aid and fire response.**
- **Incidents should be reported to the worker's supervisor.**

- **Supervisors should file incident reports with input from the individuals involved.**
- **All incidents should be reported to the CDRF manager and OSC safety officer.**
- **Biosafety incidents should be reported to the BSO.**
- **A record of all emergencies is to be kept by the Facility Manager.**
- **In an emergency situation, personal safety takes precedence over maintaining containment.**

Contact information

- **Call 911 for urgent fire and medical emergencies.**
  You must still dial 9 for outbound calls, i.e. 9-911.
- **Call campus enforcement and patrol for non-urgent problems: 864-4100.**
- Report all incidents to your supervisor and the facility manager.
- **CDRF manager: Stephen Hill: 709-864-3258**
  Containment or building wide problems. Biohazard incident reporting.
- **Institutional Biological Safety Officer (BSO): Rod Hobbs: (709) 864-8250**
  Main university contact for federal and provincial biosafety legislation, including CFIA regulations. Issues permits for pathogen work at the university and must be contacted before beginning work with a new biohazardous organism.
- **Containment RA: Gord Nash: 709-864-2722**
  Containment lab specific issues including animal care. Water quality, including problems with Aquabiolab tanks, water treatment and deep-sea equipment.
- **OSC Laboratory Services Supervisor: John Evely: 709-864-3707**
  Building infrastructure and equipment failures: electrical, water, damage, etc. OSC safety officer: Connie Short: 709-864-3223
- **Marsha Roche: 709-864-3709**
  Local lab and fire safety questions and incident reporting. Member of Institutional Biosafety Committee.

Incident reporting

All work-related incidents, injuries or occupational diseases (including potential LAI's) must be reported to Memorial's Incident Management System (MIMS) within 24 hours by following the link below:

http://www.mun.ca/MIMS/

The incident will be reviewed and the safety SOPs amended to account for the incident with new procedures aimed to avert it in the future. Near miss incidents should also be reported.

Fire and natural disaster

Responding to a fire emergency in the containment zone must balance personal safety and the need to retain containment. Personal safety always takes precedence in a true emergency. Presently exit and entrance procedures follow AQC3 guidelines.

1. **If you discover fire, smoke or smell gas, follow the evacuation procedures and evacuate immediately from the nearest exit.**
2. **If there is a fire alarm with no signs of gas, smoke or fire (a potential false alarm), immediately stop what you are doing and exit following normal biocontainment procedures.**
    a. **Leave BSC running with contents inside.**
    b. **Doff lab coats and gloves.**
    c. **Use hand sanitizer on hands.**
    d. **Do not leave the emergency gathering point.**
    e. **Upon return to the CDRF, properly decontaminate footwear and hands.**
3. **Fire drills will be announced ahead of time. Stop all experiments before the drill and exit in the usual manner following all containment procedures.**

Medical emergency

1. **Call 911 or campus patrol as appropriate.**
2. **Perform first aid as needed.**
3. **If able to do so, exit the CZ with the injured following containment procedures.**
4. **If remaining in the CZ, prepare a path for first responders, clear of experiments and enlist help to guide them in.**
5. **Emergency responders may follow reduced containment protocols, but should be informed of the status. If possible, they should use the foot baths and containment entry and exit points.**

Small wounds

There are many situations within the lab where small cuts, scrapes or skin puncturing wounds may occur, typically on the hands. This includes accidents with needles, scalpels and animals (bites and scratches). For this reason, double gloves should be used when handling infectious materials including live animals or the water they are in. In the event of a small wound that breaks the skin, follow these steps:

1. **Remove gloves or other PPE covering the wound in an area away from infectious material, animals and water.**
2. **Wash the wound with flowing tap water and clean with antiseptic wipes from the first aid kit.**
   First aid kits are located in all laboratories.

3. **Cover the wound with dressing or bandages from the first aid kit.**
4. **Re-don gloves and PPE.**
5. **Report the incident to the BSO and complete an incident report form.**
6. **Review the pathogen safety data sheet for information on the symptoms of infection in humans (if any) and monitor yourself for these symptoms.**

Post-exposure response

1. **Emergency first aid/Reporting**
   If first aid is required, it should take precedence over everything else. If emergency first aid is not required, immediately inform your supervisor of the details of the exposure. The supervisor is required to submit an incident report outlining the details of the exposure.
2. **Medical testing/treatment**
   As soon as possible, report to the emergency department of the closest local hospital for prescribed testing/treatment.
3. **Follow-up**
   The BSO will follow up with the individual exposed and his/her supervisor to review the incident. During this follow-up, a root cause of the exposure will be identified and preventative measures put in place to minimize the likelihood of reoccurrence.

Power and BSC failure

In the event of a power failure the OSC has back up power which should activate within a few seconds of the initial power failure. If the backup power fails, the following actions should be taken.

1. **Stop all experimental activity. Close all containers, put out burner flames.**
2. **If operating in the BSC, close all open containers and close the sash.**
   a. **Upon return of power allow BSC to run for 30 minutes before resuming operations.**
3. **So far as possible, follow procedures for a WWTP failure (see below).**
   This will entail a minimal version of that protocol, since not all equipment will function. The chief concern is to stop water flow.
4. **Emergency lights will be on. Exit in the usual manner. Additional flash lights can be found within the CZ if needed.**
5. **Before re-entering when the power returns, be sure that negative pressure has returned.**
   Survey animals and experiments for animal care and biosafety concerns. Take special note of any issues with temperature of water, freezers, fridges and incubators.

WWTP failure

A failure in the wastewater treatment may include leaks, equipment failure or below threshold reading on treatment processes such as UV intensity. In these events, both incoming and outgoing water flow is stopped. This may happen automatically or be done manually by the water quality technician or facility support staff.

This will necessitate turning running flow through tanks and systems into static aquaria with the addition of oxygen bubblers.

Conditions that trigger WWTP alarms

Under the following conditions an audible alarm will sound and a visual alarm will display on panel CP 5.1. An alarm condition will be reported at the custodian's desk. See appendix A for a diagram of the WWTP process.

- **Discharge pump failure.**
- **Collection tank high water level pressure transducer alarm.**
- **Collection tank high water level float switch alarm (backup to pressure transducer).**
- **Either UV system is off or below UV transmission threshold.**
- **Detection of flooding in the pit.**

Response to WWTP alarms

1. **There is no need to stop water flow as the system will automatically close valves to incoming and outflowing water.**
   Confirm that water flow has halted.
2. **Inform the CZ RA or CDRF manager of the alarm.**
   They will contact laboratory services for assistance (ext. 3236 or 3707).
3. **Follow the animal care protocol for ongoing experiments to maintain water quality until the emergency is resolved.**
   This will likely entail maintaining tank oxygen levels with air stones and monitoring water quality parameters. In the event of a severe WWTP failure, animals may need to be euthanized if water quality cannot be maintained. They may not be relocated outside of the CZ.

Testing of automated systems

The automation of WWTP shut-down in the event of a failure condition will be tested annually if no events have occurred in the year.

1. **Manually shut down one of the UV lamps.**
2. **Confirm that an alarm state is registered on CP5.1 and that incoming flow is halted in room CD-1010. Confirm that UVs in room CD-1010 shut down.**
3. **Restart the UV lamp. Once it has warmed up, confirm that flow resumes.**
4. **Lift and tilt the high water float switch in the collection tank.**
5. **Repeat step 2.**
6. **Return float valve to normal resting point and confirm that the system restarts.**

Exhaust fan failure

Failure of the exhaust fans may result in the release of aerosol and infectious material outside of the containment zone. This will be accompanied by a loss of negative pressure in the CZ.

1. **If the exhaust fan fails, or negative pressure is lost, an alarm will sound at the negative pressure alarm by the CZ entrance.**
2. **The remote annunciators will sound and the red light will come on.**
   The remote annunciator has a battery backup and may be temporarily relocated to other rooms. Return to room CD-1022 and plug in under normal operation.
3. **Immediately stop all experimental procedures. Close open containers in the BSC. Close all tank lids.**
4. **Notify the CDRF manager or CZ RA.**
   They will contact laboratory services for assistance (ext. 3236 or 3707).
5. **Exit using normal biocontainment procedures.**
   Do not resume experiments in the CZ until the air pressure has been resolved.

Remote annunciator. Green light solid when on, flashes when connected to transmitter. Red light and buzzer when main air pressure monitor is in alarm.

General Spill Clean-up Procedure

All following spill procedures are adapted from WHO (2004). When a spill occurs, the worker should determine if it is a small or large spill and follow the appropriate procedure below. “Small spills” are easily contained from spreading with paper towel and have not contaminated the clothing of any personnel. “Large spills” are those that are not easy to immediately contain from spreading and / or have contaminated the clothing of any personnel.

1. **Wear gloves and protective clothing, including eye protection.**
2. **Bring spill kit to the site of the spill.**
   Spill kits are located by the emergency showers in rooms CD-1011, 1022 and 1023.
3. **Cover the spill with cloth or paper towels to contain it.**
4. **Pour an appropriate disinfectant over the paper towels and the immediately surrounding area**
   Generally, 5% bleach solutions are appropriate. Apply disinfectant concentrically beginning at the outer margin of the spill area, working toward the center. Spills kits containing bleach solutions should be refreshed monthly.
5. **After the appropriate contact time (e.g. 30 minutes), clear away the materials.**
   If there is broken glass or other sharps involved, use a dustpan or a piece of stiff cardboard to collect the material and deposit into a puncture resistant container for disposal. Glass fragments should be handled with forceps. Dustpans should be autoclaved or placed in an effective disinfectant.
6. **Clean and disinfect the area of the spillage – if necessary repeat the steps above.**
7. **Dispose of contaminated materials into a leak-proof, puncture-resistant waste disposal container.**

8. **After successful disinfection, inform the facility manager or containment RA that the site has now been decontaminated.**
   If indicated by the pathogen risk assessment, the operator will be referred to medical attention. The facility manager will keep a record of spills for BSO audit.

Small spills – inside the BSC

Worker is not considered contaminated unless actual splash or spillage onto their person occurred.

1. **Gloves and sleeves are considered contaminated. These should be removed prior to removing hands from BSC. Fresh gloves should be donned and if necessary a fresh lab coat should be donned.**
2. **BSC should not be turned off, nor should the sash be lowered.**
3. **Follow General Spill Clean-up Protocol.**

Large spills – inside the BSC

Worker is assumed to be contaminated.

1. **Outer layer of protection is considered contaminated. This should be removed at the BSC.**
   Place on floor beside BSC. Inner layer of protection is not considered contaminated unless actual splash occurred.
2. **Personnel should vacate the laboratory for an appropriate period of time to allow BSC to filter the contaminated air and to allow aerosols to settle.**
   Allow at least 30 min for this.
3. **If inner layer must be removed, the worker should proceed to a full body shower including hair.**
   Clothing must be collected for sterilization. Scrubs / clean room apparel are available as temporary clothing.
4. **The facility manager or containment RA should be informed at once.**
5. **Appropriate PPE must be worn on re-entry.**
6. **Follow General Spill Clean-up Protocol. The entire surface of the BSC, including the work surface, the sides, back and interior of the glass and the catch pan, must be decontaminated.**
   The catch pan must be flooded with an appropriate disinfectant allowing an appropriate contact time prior to removing the work surface to clean the catch pan.
7. **Never should the head enter the BSC during decontamination.**
8. **All items inside the BSC at the time of the spill must be decontaminated.**
   Contaminated clothing to be placed in bin for laundry sterilization.

Large spills – outside the BSC

This includes spills where the inner layer of clothing has become contaminated.

1. **The worker removes the outer layer of protection next to spill.**

2. **The worker proceeds to the dirty change room and removes all items of inner layer of protection and places them in an autoclave bag. While still wearing inner gloves, the worker decontaminates eye glasses. The worker proceeds to full body shower including hair.**
   Scrubs are available to use as replacement clothing.
3. **The containment RA and facility manager should be informed at once.**
   The BSO should also be informed ASAP.
4. **Re-entry into the laboratory should be restricted for an appropriate amount of time (e.g. 1 hour) to allow enough air exchanges to clear away the aerosols and to allow heavier particles to settle.**
5. **Signs should be posted indicating that entry is forbidden.**
6. **Once spill is cleaned up as per the general spill clean-up procedure, personnel must doff PPE in the dirty change room and don clean PPE prior to returning to work in the laboratory.**
7. **Depending on the nature and size of the spill, a full room decontamination may be warranted.**

Spill inside a centrifuge in a sealed safety cup or sealed rotor

1. **All sealed centrifuge buckets should be loaded and unloaded in a BSC.**
2. **If breakage is suspected within the safety cup, the safety cap should be loosened and the bucket autoclaved.**
   Alternatively, the safety cup may be chemically disinfected.

Spill inside a centrifuge - infectious material visible upon opening the centrifuge

1. **If a breakage occurs or is suspected while the machine is running, the motor should be switched off and the machine left closed (e.g. for 30 minutes) to allow settling.**
2. **If a breakage is discovered after the machine has stopped, the lid should be replaced immediately and left closed (e.g. for 30 minutes).**
3. **Inform the containment RA and facility manager.**
4. **Appropriate PPE must be donned prior to opening the centrifuge.**
   Lab coat, gloves, eyeglasses and surgical mask or face shield.
5. **All personnel not involved in the spill clean-up must exit the area of the spill.**
6. **Spill should be covered in absorbent towels and a non-corrosive disinfectant known to be active against the organism concerned should be poured over the spill being cautious not to create aerosols.**
7. **All broken tubes, glass fragments, buckets, trunnions and the rotor should be placed in a noncorrosive disinfectant.**
   Forceps must be used to handle and retrieve glass debris.
8. **Unbroken sealed safety cups may be placed in disinfectant and carried to a BSC to be unloaded.**

9. **The centrifuge bowl should be swabbed with the same disinfectant, at the appropriate dilution, and then swabbed again, washed with water and dried.**

References

1. *Laboratory biosafety manual*. (World Health Organization, 2004).

2. *Containment Standards for Facilities Handling Aquatic Animal Pathogens*. (Canadian Food Inspection Agency, Biohazard Containment and Safety Science Branch, 2010).

3. *Canadian Biosafety Standards and Guidelines*. (Government of Canada, 2015).

Version history and notes

Version	Date	Authors	Notes
1.0	2014-04-01	Stephen Hill	First writing [1–3]. CFIA first submission
2.0	2014-07-25	Stephen Hill	Edits from first review by CFIA. Clarification of spill procedures.
2.1	2014-09-22	Stephen Hill	Addition on WWTP and air flow failures.
2.2	2015-03-17	Stephen Hill	Edits from Rod Hobbs.
2.3	2015-05-05	Stephen Hill	Minor clarifications. Pre-submission.
2.4	2015-12-09	Stephen Hill	Revision of incident response and reporting as per MUN biosafety manual.
3.0	2016-01-14	Stephen Hill	Updating WWTP failure plan.
3.1	2016-12-19	Stephen Hill	Minor corrections.
3.2	2017-04-08	Stephen Hill	Added information about remote annunciator. **CFIA approved 2017.**
4.0	2018-05-02	Stephen Hill	Minor changes, updates to contacts. Pre-CFIA 2018 resubmission.

Appendix A: WWTP process diagram