



Standard Operating Procedure ***for the Collection & Disposal of Waste in Clean Zone***

DOUCMENT PARTICULARS	
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Collection & Disposal of Waste in Clean Zone**Review History**

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01	09 Jan 2023	01 Feb 2023	01 Aug 2023	New SOP

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Collection & Disposal of Waste in Clean Zone

A) Objectives

This document describes the procedures used by member of *Centre for Regenerative Medicine and Health, Hong Kong Institute of Science & Innovation, Chinese Academy of Sciences Limited* (refer to CRMH below) to handle wastes generated in the cleanroom to final disposal and destruction.

B) Scope

The SOP applies to all wastes generated in the clean zone.

C) Facility Covered

Clean zones in CRMH, 5/F, 15 Science Park West Avenue, Hong Kong Science Park, Pak Shek Kok, Hong Kong

D) Responsibilities

1. Team manager provides the training of this SOP to their members and to oversees if their members properly follow the waste collection & disposal procedures.
2. The team manager and designated person(s) has the following responsibilities
 - 2.1. Manages supplies, biohazard bags, sharp containers, autoclave bags and the supply of disinfectants within the cleanroom.
 - 2.2. Collects biological waste in suitable containers.
 - 2.3. Segregates biological wastes from other wastes.
 - 2.4. Organises the treatment of waste.
 - 2.5. Ensure suitable label(s) is attached.
 - 2.6. Refer to centre guidelines for autoclave operations.
3. Every member working in the clean zone should diligently adhere to the established clean room SOPs and inform the team manager if deviations occur.
4. F&OC provides necessary support to the team managers (or their delegates) to implement this SOP.

E) References

1. International Organisation for Standardisation. *Cleanrooms and associated controlled environments – Part 5: Operations*. ISO 14644-5: 2004, 2004.
2. World Health Organisation. *Laboratory Biosafety Manual, 4th Ed.* 2020.

F) Definitions:

1. **Biohazardous spills** are spills that contain any biohazardous agents.
2. **Biohazardous agents** are any agents that are biological in nature and have the capacity to produce harmful effects upon other biological organisms. Biohazardous agents include, but not limited to:
 - i. Bacteria
 - ii. Viruses
 - iii. Fungi
 - iv. Cell lines
 - v. Animal tissues
 - vi. Clinical specimens
 - vii. Recombinant nucleic acid products
 - viii. Toxins of biological origin

3. **Chemical spills** are waste that is derived from chemicals that have not been in touch with biological agents
4. **Sharps wastes** are discarded articles that may cause punctures or cuts, including but not limited to needles, syringes, pipettes etc.

Names of rooms/areas of a typical cleanroom complex in CRMH:



1. Biological waste containers, with a lids
2. Autoclave bags
3. Sharps box
4. Liquid biological waste container
5. Chemical waste container
6. Autoclaves location: 1/F, 15W, HKSTP

1. ***Solid biological waste collection & disposal (For general cell culture)***
 - 1.1 Method A (Dispose of the solid biological waste directly in the autoclave bag)
 - 1.1.1 Solid biological waste includes all non-liquid, non-sharp wastes which are contaminated with biological agents.
 - 1.1.2 Obtain biological waste bins and autoclave bag, the waste bin and the autoclave bag must both be labelled clearly, with a biohazard symbol and the word “Biohazard”
 - 1.1.3 Line the waste bin (with cover) with autoclave bag.

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- 1.1.4 Dispose solid biological waste into the waste bin as it is generated. Serological pipettes should be returned to their packet before putting into the bin.
- 1.1.5 When the autoclave bag (in waste bin) is $\frac{3}{4}$ full, zip-tied or taped close the bag and remove it from the bin.
- 1.1.6 Autoclave bag with biological waste should always be tied before translocation.
- 1.1.7 Autoclave tape should be stuck onto the bag surface for identification.
- 1.1.8 Place a new autoclave bag inside the bin.
- 1.1.9 Move the autoclave bag (with waste) to a dedicated metallic tray inside the clean room for temporary storage.
- 1.1.10 The tied autoclave bag (with waste) is moved to the designated area in open laboratory following the *CRMH-SOP-003: SOP for Material Entry & Exit of Clean Zone*. Solid biological waste described here must be autoclaved prior to disposal as municipal solid waste.
- 1.1.11 All waste bags are cleared out of the clean zone every morning before work starts.
- 1.2 Method B (Dispose of the solid biological waste pre-treated with bleach solution)
 - 1.2.1 Prepare fresh 1:50 bleach solution.
 - 1.2.2 Consumable such as serological pipettes/ culture flask/ dishes/ plates should be disinfected by rinsing with 1:50 bleach solution. The serological pipettes should be disinfected by pipetting up and down the bleach solution.
 - 1.2.3 After discarding all the remaining bleach solution, the consumables can be discarded as domestic waste in the domestic waste bin inside the clean room.
 - 1.2.4 (Optional) For the disinfected serological pipettes, put them in a secondary container, which are transferred to the open laboratory right after the experiment following the *CRMH-SOP-003: SOP for Material Entry & Exit of Clean Zone*. Dispose the disinfected serological pipettes to a domestic waste bin.
 - 1.2.5 When the domestic waste bag (in waste bin) in the clean room is $\frac{3}{4}$ full, zip-tied and remove it from the bin.
 - 1.2.6 Place a new domestic waste bag inside the bin.
 - 1.2.7 Move the domestic waste bag (with waste) to a dedicated metallic tray inside the clean room for temporary storage.
 - 1.2.8 The tied domestic waste bag (with waste) is moved to the designated area in open laboratory following the *CRMH-SOP-003: SOP for Material Entry & Exit of Clean Zone*.

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1.2.9 All waste bags are cleared out of the clean zone every morning before work starts.

2. *Liquid biological waste collection & disposal (For general cell culture)*

- 2.1 Liquids include tissue culture media, cell supernatant, or any other liquid generated from biological agents.
- 2.2 Liquid waste is collected using labelled waste flasks or beakers that can be easily covered. Check for leaks before using the container.
- 2.3 Label the container as "Liquid biological waste".
- 2.4 Active liquid waste containers must be kept in a secondary plastic tray.
- 2.5 Procedure for using waste beaker/ bottle as the liquid biological waste container
 - 2.5.1 Pre-fill a liquid waste container with household bleach which is 1/10 of the max. volume of the container. More than 1 waste containers are required when large waste volume would be generated.
 - 2.5.2 Transfer the waste solution into the liquid waste container. When the liquid waste container is 2/3 full in an ongoing experiment, seal the container and put it aside of the BSC until finishing the experiment.
 - 2.5.3 To continue the experiment, transfer the newly generated waste solution to another liquid waste container (with household bleach which is 1/10 of the max. volume of the container that has been already prepared in procedure 2.5.1.).
 - 2.5.4 When the experiment is finished, seal all the containers and transferred them outside the clean zone to complete the disinfection.
 - 2.5.5 Complete the disinfection process by incubating the waste solution with household bleach at least 30 mins (from start) before discarding in domestic drainage. The disinfection process should be conducted in a running fume hood.
- 2.6 Procedure for using waste flask connected with cell culture vacuum pump
 - 2.6.1 Make sure the vacuum pump has equipped with a HEPA filter
 - 2.6.2 Pre-fill a liquid waste container with household bleach which is 1/10 of the max. volume of the container. More than 1 waste containers are required when large waste volume would be generated.
 - 2.6.3 Transfer the waste solution into the liquid waste container. When the flask (connect to the vacuum pump) is 2/3 full in an ongoing experiment, transfer the liquid waste to the container of step 2.6.2.
 - 2.6.4 Re-install the flask to the vacuum pump again to continue to collect the liquid waste.
 - 2.6.5 When the experiment is finished, transfer all liquid waste stored at the flask (connect to vacuum pump) to the waste container.

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- 2.6.6 Rinse the tubing connected to the flask with 1:50 bleach solution to remove the residual culture medium.



- 2.6.7 When the experiment is finished, seal all the waste containers and transfer them outside the clean zone to complete the disinfection.
- 2.6.8 Complete the disinfection process by incubating the waste solution with household bleach at least 30 mins (from start) before discarding in domestic drainage. The disinfection process should be conducted in a running fume hood.
- 2.7 Beware of chemical reactions between the chemicals in the waste and the disinfectant. If unsure, check MSDS or consult team manager & F&OC. For example, ethanol should not be mixed with bleach.
- 2.8 The final concentration of bleach solution must exceed 0.5% sodium hypochlorite (No less than 1 part household bleach to 9 parts liquid waste). This final concentration can be achieved by adding household **bleach inside fume hoods in open lab**.
- Caution: Diluted bleach solution used must be freshly prepared in the same day.*
- 2.9 Transfer the waste mixture out of the clean zone daily following “CRMH-SOP-003: SOP for Material Entry & Exit of Clean Zone”.
- 2.10 Liquid waste should be disinfected / incubated with household bleach for at least 30 minutes (from start).
- 2.11 After 30 minutes, pour the waste mixture into the sink with running tap water in the fume hood.
- Remark: Liquid waste must be treated with bleach and must not be left untreated overnight.
- 2.12 If the container is to be left unattended during disinfection, the container should be labelled with the start time at which household bleach is introduced and kept in the plastic tray.

3. Biological sharps waste collection & disposal

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- 3.1 Any item that can penetrate the skin are considered to be sharps such as glass pasture pipettes, scalpels, needles, blades, broken glass.
- 3.2 All sharps, after use, should be immediately dropped into a sharps box with biohazard label.



- 3.3 When waste has been filled up to the red line, carefully close the container with the top cover.
- 3.4 Transfer the waste out of the clean zone following "CRMH-SOP-003: SOP for Material Entry & Exit of Clean Zone".
- 3.5 Precautions for sharps disposal:
 - 3.5.1 Make sure the sharp tip is completely inside the sharps box.
 - 3.5.2 Do NOT apply an extra force to push sharps into a container.
 - 3.5.3 Do NOT recap needles or remove needles from syringes before disposal.
 - 3.5.4 Do NOT attempt to dismantle a sharps box with sharps waste inside, or to open the cover of a full sharps box.
- 3.6 Put the closed sharps box at collection point, located next to fume cupboard (see below). Sign the log sheet to record your disposal.



- 3.7 F&OC will arrange regular collection of sharps boxes.
4. *Adenovirus/ Adeno-associated virus/ Lentivirus contaminated waste*
 - 4.1 Waste contaminated with Adenovirus/ Adeno-associated virus/ lentivirus are collected separately.
 - 4.2 Refer to CRMH-SOP-008: SOP for Adenovirus/ Adeno-associated virus/ Lentivirus Handling Safety and Risk Management in Clean Zone.
5. *Chemical waste collection and disposal*

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- 5.1 Equip appropriate powder-free gloves to handle the chemical waste which has not contaminated with biological agents.
- 5.2 Collect the chemical waste with a suitable container.
- 5.3 Place the chemical waste container into a basket.
- 5.4 Wipe the basket with 70% ethanol for the materials transfer.
- 5.5 Wastes containing halogenated chemicals or UV sensitive chemicals should be placed into a secondary container to protect it from UV. This procedure is applicable only when UV light sterilization is needed during the transfer in passbox.
- 5.6 Follow “CRMH-SOP-003: SOP for Material Entry & Exit of Clean Zone” for exporting the waste container in basket to open lab through passbox.
- 5.7 Treat the chemical waste according to suggestions in MSDS, and place the chemical waste to the corresponding chemical waste pail in the open laboratory.
- 5.8 F&OC will arrange authorized chemical waste collector to collect the chemical waste.

J) Abbreviations

1. BSC: Biosafety cabinet
2. F&OC: Facilities and Operations Compliance
3. MSDS: Material safety data sheet
4. PPE: Personal protective equipment
5. SOP: Standard operating procedure