中國科学院香港創新研究院
再生医学与健康創新中心
Centre for Regenerative Medicine and Health
Hong Kong Institute of Science & Innovation, Chinese Academy of Sciences

# Standard Operating Procedure

## *for Adenovirus/ Adeno-associated virus/ Lentivirus Handling Safety and Risk Management in Clean Zone*

| DOUCMENT PARTICULARS | |
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## Review History

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| 01 | 09 Jan 2023 | 01 Feb 2023 | 01 Aug 2023 | New SOP |
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## A) Objectives

1. This document describes the proper procedures for safe handling of adenovirus/ adeno-associated virus/ lentivirus used by CRMH members in the clean zone. The clean zones locate at of the Centre for Regenerative Medicine and Health, Hong Kong Institute of Science & Innovation, Chinese Academy of Sciences Limited (refer to CRMH below).
2. This document describes the risk management practices for adenovirus/ adeno-associated virus/ lentivirus.

## B) Scope:

The SOP applies to all personnel who handle the adenovirus/ adeno-associated virus/ lentivirus 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

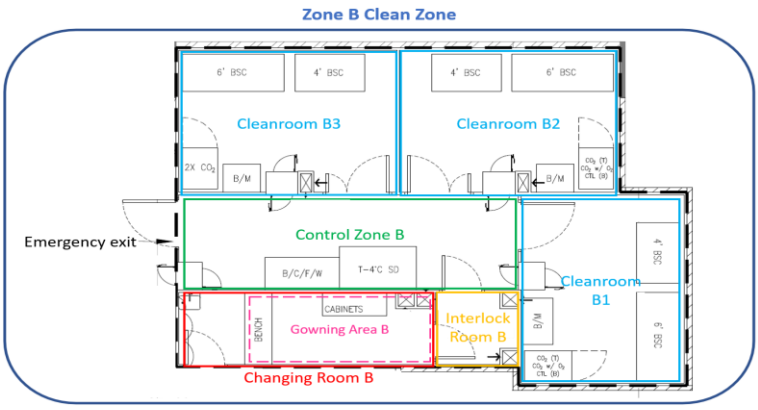
1. Team manager provides the training of this SOP to their member and to oversees if their member properly follows the safety procedures and risk management in handling the adenovirus, adeno-associated virus, or lentivirus.
2. Team manager allocates designated clean room, BSC, incubator for cell culture with adenovirus, adeno-associated virus, or lentivirus.
3. The personnel entering 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 the SOPs.

## E) References

International Organisation for Standardisation. *Cleanrooms and associated controlled environments – Part 5: Operations*. ISO 14644-5: 2004, 2004.

## F) Nomenclature:

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




Floor plan of a typical Clean Zone in CRMH.

## G) Procedures:

1. *Risk Management against Lentivirus/ Adeno-associated virus/ Adenovirus*

   *1.1.* The experiment involves Lentivirus/ Adeno-associated virus/ Adenovirus must be assessed the associated risk(s) and approved by team manager prior to commencement of work.

   *1.2.* Documents including but not limited to the MSDS of the virus, protocol and assessment of the experiment have to be kept by team managers at least 1 year after the completion of the project. Copies of these documents are required to provide to the F&OC and CRMH safety committee for retention.

2. *Physical containment regarding work with Adenovirus/ Adeno-associated virus/ Lentivirus*

   2.1. All work concerning adenoviruses, adeno-associated virus, or lentiviruses must be performed in a certified Class II BSC inside the designated clean room.

   2.2. Centrifugation must be done in closed containers and using sealed rotors or safety cups.

   2.3. Safety cups should only be opened inside the BSC.

   2.4. All vacuum lines must be fitted with a HEPA filter.

   2.5. Cell culture contaminated with adenoviruses, adeno-associated virus, or lentiviruses can only be incubated in the designated incubator.

   2.6. Inside the incubator, the culture dishes or flasks contaminated with adenoviruses, adeno-associated virus, or lentiviruses must be kept in a secondary container to avoid that culture medium leaking from the culture dishes or flasks and contaminating the incubator.

3. *General procedures regarding work with Adenovirus/ Adeno-associated virus/ Lentivirus*

   3.1. Standard BSL-2 practices must be employed, please refer to “CRMH-SOP-001: SOP for General Good Safety Practices in Clean Zone”.

   3.2. Appropriate PPE must always be equipped.

   3.3. Biohazardous Spill Kits must be present and easily accessible.

   3.4. The “Risk assessment sheet” (see Appendix 3 of CRMH-SOP-003) of the experiment has to be submitted to the team manager. The team manager has to assess the associated risk(s) of the experiment involving lentivirus/ adeno-associated virus/ adenovirus. The experiment can be commenced in the clean zone only when approval from team manager is obtained.

   3.5. Do **NOT** work with lentivirus, adeno-associated virus, or adenovirus containing materials outside the designated clean room.

3.6. Bring all necessary materials into the designated clean room before the start of work.

3.7. Only replication incompetent transfer systems can be used.

3.8. It is recommended NOT to use glass or needles for work involving adenovirus, adeno-associated virus, or lentivirus.

3.9. It is recommended to use double gloves when handling adenovirus, adeno-associated virus, or lentivirus.

3.10. Outer layer of PPE should always remain in the designated clean room.

3.11. Outer layer of PPE should be carefully packaged according to the "De-gowning SOP", then transferred to the autoclave and decontaminated prior to laundry. The autoclave must be done at the 1/F, 15W of HKSTP.

3.12. A secondary container with a lid should be used to transfer containers with viral material within the clean zone.

3.13. Signs and labels must be placed to indicate the area where the adenovirus, adeno-associated virus, or lentivirus is being used or stored. Signs must include but not limited to the name of the agent, emergency contact information, and a biohazard sticker.

3.14. A designated incubator, BSC, and clean room should be reserved for the handling of adenovirus, adeno-associated virus, or lentivirus.

3.15. Always disinfect the BSC using 70% ethanol and UV for 30 mins after work.

4. *Decontaminating consumables after contact with adenovirus/ adeno-associated virus/ lentivirus*

4.1. Prepare fresh 1:50 bleach solution.

4.2. Consumable such as serological pipettes, culture flask/ dishes/ plates should be primarily disinfected by **rinsing with freshly prepared 1: 50 bleach solution** from the container in Step 4.1.

4.3. After pre-treatment, the consumables that have contact with adenovirus/ adeno-associated virus/ lentivirus should be put into **designated waste container** separated from normal biological wastes.

4.4. All adenovirus/ adeno-associated virus/lentivirus-contaminated wastes must be double bagged and transferred out from the clean zone to the designated waste collection point of open lab **on the same day** following the *CRMH-SOP-003: SOP for Material Entry & Exit of Clean Zone*.

4.5. F&OC will arrange disposal by an authorized waste collector**.**

5. *Decontaminating solution containing adenovirus/ adeno-associated virus/ lentivirus*

5.1 Pre-fill a liquid waste container with 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.

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.

5.3 Transfer the waste solution into another liquid waste container that has been already prepared in procedure 5.1.

5.4 When the experiment is finished, seal all the containers and transferred them outside the clean zone for further disinfection after experiment.

5.5 Further disinfection should be carried out in a running fume hood.

5.6 Ensure the solution incubate with bleach for 30 mins before discarding in domestic drainage.

6. *Handling of spills concerning adenovirus/ adeno-associated virus/ lentivirus*

Refer to the CRMH-SOP-007: SOP for Spills and Exposures in Clean Zone

7. *Incidents and exposure events*

7.1. Exposure to skin or mucous membrane

7.1.1. Stop current work.

7.1.2. Rinse the exposed area with 1 bag of IV grade saline.

7.1.3. De-gown and leave the clean zone through changing room (use the emergency exit in control zone for urgent cases).

7.1.4. Rinse exposure area with water for at least 15 mins.

7.1.5 Report the incident according to the *CRMH-SOP-007: SOP for Spills and Exposures in Clean Zone.*

7.2. Penetrating wound

7.2.1. Contaminated skin should be thoroughly scrubbed for several minutes with 1 bag of IV grade saline or a 10% povidone solution.

7.2.2. De-gown and leave the clean zone through changing room (use the emergency exit in control zone for urgent cases).

7.2.3. Rinse the wound with water for at least 15 mins.

7.2.4. Notify the team manager and F&OC.

7.2.5. Seek medical attention.

7.2.6. Report the incident according to the *CRMH-SOP-007: SOP for Spills and Exposures in Clean Zone*.

7.3 First aid

7.3.1. Call emergency service (999) or press the Emergency Call Button to inform Hong Kong Science and Technologies Parks Corporation (HKSTP) if immediate medical care is needed.

7.3.2. Stabilize the individual and provide first aid for injuries that require immediate medical care (e.g. bleeding).

7.3.3. Notify the team manager and F&OC after the individual has been stabilized.

7.3.4. Report the incident according to the *CRMH-SOP-007: SOP for Spills and Exposures in Clean Zone*.

8. *Replication competent virus (RCV) testing*

8.1 Adenoviruses/ Adeno-associated virus

8.1.1. Viral preparations can be heat-inactivated for 15 mins at 56°C and tested for the presence of replication competent adenovirus by plaque assay or cytopathic effect.

8.2 Lentiviruses

8.2.1. First and second generation lentiviral systems require RCV testing.

8.2.2. Lentivirus vectors can be tested for RCV by serial transfer and ELISA for p24 antigen.

8.2.3. Vectors used for in vitro studies must be tested every 6 months.

9 *Storage of samples containing Adenoviruses/ Adeno-associated virus/ Lentiviruses*

9.1 Samples should be stored with a secondary container following *CRMH-SOP-003 Material Entry & Exit of Clean Zone*

9.2. The container should be leak-and puncture-proof.

9.3. A clear label with a biohazard symbol  and “Adenovirus/ Adeno-associated virus/ Lentivirus” should be labelled on the container.

## H) Abbreviations

1. BSL-2: Biosafety level 2
2. BSC: Biosafety cabinet
3. F&OC: Facilities and Operations Compliance
4. HKSTP: Hong Kong Science and Technologies Parks Corporation
5. PPE: Personal protective equipment
6. RCL: Replication competent lentivirus
7. RCV: Replication competent virus
8. SOP: Standard operating procedure