Document Number: **SASoM/METHOD/009.v5**

Title: **Generating and working with viral particles at Biosafety Level 2 (BSL2)**

Version: **v5**

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SOP History		
Number	Date	Reason for Change
V1	01/01/2013	Original
V2	01/01/2015	Update
V3	01/01/2017	Update
V4	01/01/2019	Update
V5	01/01/2021	Update

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1.0 Purpose –

The purpose of this SOP is to outline the principles and safety issues for the generation and use of replication deficient, non-mobilisable, viral particles (retrovirus and lentivirus at Biosafety / Containment Level 2) for use in transducing mammalian cells in Laboratory 248 at the St Andrews School of Medicine (SASoM).

2.0 Scope –

This SOP applies to generation and use of replication deficient, non-mobilisable, viral particles (retrovirus and lentivirus at containment level 2) for use in transducing mammalian cells within the SASoM.

3.0 Responsibilities –

It is the responsibility of all users carrying out viral work within the SASoM to comply with this SOP. Under no circumstances should viral work be undertaken by anyone who is not acquainted with it or has not been trained. All users must read and then sign the SOP before using it and comply with all safety instructions.

4.0 Procedure –

Viral work should only be carried out in the biosafety cabinet in 248K labelled as "Viral" (furthest away from door). No glass or sharps are allowed inside the cabinet, except for a glass bottle containing freshly prepared 1% virkon. Avoid the generation of aerosols, which would release viral particles into the air of the cabinet. No viral-contaminated solid waste to leave the cabinet except in a sealed plastic bag. No viral-contaminated liquid waste to leave the cabinet except aspirated by the aspirator line from inside the cabinet, which should then be immediately flushed with 1% virkon. Spills should be immediately absorbed on white tissue and added to solid waste, the area cleaned with 1% virkon and then 70% ethanol.

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Preparation and Safety Checks:

1. Remove the UV light box and ensure that the biosafety cabinet is working properly with correct airflow.
2. Ensure the vacuum pump is on under the cabinet and that the liquid waste bottle is less than two-thirds full. Otherwise seek assistance.
3. Place an open plastic autoclave bag inside the cabinet (for viral contaminated solid waste), placed carefully in the centre of the cabinet so as not to disturb the cabinet airflow.
4. After wiping its outside with 70% ethanol, place a bottle of freshly prepared 1% virkon solution (virkon powder in water) inside the cabinet.
5. After wiping with 70% ethanol, arrange electronic pipettor, racks and plasticware neatly so as this doesn't affect correct airflow of cabinet.

Generation of viral particles:

6. For retrovirus, producer cell lines may be used that contain incorporated packaging plasmids e.g. Phoenix A cells. For lentivirus, packaging plasmid must be introduced for each experiment – never incorporate lentiviral packaging plasmids in the producer cells for long-term propagation beyond the current experiment.
7. Once producer cell lines contain packaging and expression plasmids (and are able to generate viral particles) they should be worked on in the "viral" cabinet.
8. Use 2mL plastic aspirating pipettes and the aspirating line and pump to remove waste cell culture media. Immediately flush the line using fresh 1% virkon.
9. Plastic pipettes and other solid waste should be carefully placed in the plastic autoclave bag, which is inside the cabinet.
10. If virus is collected at different times e.g. 48hr and 72hr, virus may be stored overnight at 4'C, but should be placed in double containment (e.g. sealed 15mL tube inside a plastic box with lid). Tubes should only be opened inside the cabinet.

Infection and culture of recipient cells:

11. Slowly add filtered virus-containing media onto recipient cells by pipetting slowly on the side of the dish, so as to avoid the generation of aerosols.
12. Incubation for the required time should be carried out in the tissue culture incubator in 248K labelled as “Viral” with the dishes placed on a plastic tray to avoid any possible spillage.
13. After incubation, dishes should be returned to the cabinet and the virus aspirated. Immediately flush the line using fresh 1% virkon.
14. Add cell culture media to the cells and incubate in the tissue culture incubator in 248K labelled as “Viral”
15. Cells should be cultured in this way until they have undergone two trypsinisation events that have moved them to new plasticware.
16. After this time, they can be considered as cells that do not pose any viral risk.

Clean up and shut down of cabinet:

17. Once work is completed, the surface of the cabinet should be sprayed with 70% ethanol and wiped down.
18. The plastic bag of viral-contaminated solid waste should be loosely sealed with autoclave tape inside the cabinet (so as to allow steam to enter during the autoclaving procedure) and placed in the large Cole-Palmer plastic solid waste containers labelled as “Viral TC”. If these are full, then seek assistance.
19. Remove all contents from the cabinet and replace the UV light box in the correct position – feet down, light facing into the cabinet and not obstructing the front airflow grill.
20. In order to remove any airborne viral particles from the cabinet, turn off the cabinet, turn it back on and wait for “mode 2” to appear on the display. Quickly press the UV light button on the panel. Press the time until “20 minutes” displays and press “Activate”. The UV light box will turn off automatically once done. The cabinet is now ready for the next user.

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5.0 Personal protection -

Howie coat specific for 248K must be worn at all times.

Gloves must be worn at all times. Gloves must be changed when operator leaves the cabinet during work. Fresh gloves are to be used on return. Contaminated gloves to be disposed into viral-contaminated solid waste bag in cabinet.

6.0 Training -

Under no circumstances should this procedure be performed by anyone who is not acquainted with it or has not been trained.

7.0 Related documents –

8.1 Procedures for tissue culture (posted on door)

8.2 Risk assessments – COSHH/004 and "working with Lentivirus" RA/BIOL/001

8.0 Approval and sign off –

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