Standard Operation procedure (SOP) for FACSVantage^{TM} SE system (FACS sorter) within the JVC BSL3 core facility

Location: 7th Floor BLSB, Room 722

Justification: The increasing need by investigators to sort cells infected with various infectious agents without fixing the cells first.

The following infectious agents may be used:

Retroviruses:

- Human immunodeficiency virus 1
- Simian immunodeficiency virus
- Lentiviral vectors

RNA viruses:

- Rabies virus (vaccination mandatory for operator)
- Vesicular stomatitis virus (New Jersey and Indiana serotypes only)
- Newcastle Disease virus (lentogenic, vaccine strains only)
- Influenza Virus (BSL2 classified strains only)

DNA viruses:

- Adenoviruses
- Vaccinia Virus (vaccination offered but not mandatory)
- Ectromelia

Of note, no infectious agent classified above BSL3 (e.g BSL4) or BLS3 requiring special operating conditions for the laboratory can be used. The list above may be extended to include novel infectious agents but only after approval by the IBC. In addition, each investigator must obtain prior approval by TJU IBC to use their respective infectious agent in the BSL3 facility. The investigator is also responsible for ensuring that the method for transporting infectious material from their laboratory to BLSB 722 follows approved guidelines. Users outside of TJU are currently not permitted to use this facility.

General information and regulations:

- The laboratory is located in Room 722 BLSB. Access to the laboratory area is restricted by TJU keycard.
- BSL3 training is mandatory to enter the laboratory
- Vaccination against vaccinia virus is optional, but rabies vaccination is mandatory.
- All personnel entering the laboratory will be informed of all infectious/viable agents used within the laboratory area as well as the potential risks associate with the agents.
- All users must read and follow the SOP form for the BSL3 laboratory and be given an introduction of its regulations by Dr. Root.

Rules for working with infectious agents or viable material at the FACS sorter

General rules: Samples can only be processed (e.g. containers/tubes with infected cells) within the shielded containment area, which provides Class II Biological Safety Cabinet-like containment (FACS-BSC, see Figure 1).

Procedure:

- Turn on the aerosol containment system 10 minutes **before** using the FACS sorter. The pressure (meter in the upper corner of FACS-BSC) must be in the range of 100-200 FPM (low speed fan).
- Open the access door to the aerosol containment chamber, and make sure that the fan switches to high speed (the door needs to be locked at the upper side of the chamber to activate the switch). The pressure must be at 150 FPM (check meter!).
- Move samples into the FACS-BSC in a closed and sealed tube
- Sort using standard procedures
- Remove sorted cells from the FACS-BSC only **after** the tube is sealed and wiped down with 10% bleach, followed by 70% Ethanol.

Decontamination procedure:

- Use a tube filled with 10% bleach to rinse the FACS system. Wait 10 minutes. Repeat once (i.e.: two cleaning steps with 10% bleach).
- Flush the system with PBS.
- Wipe all working surfaces within the FACS-BSC with 10% bleach, followed by 70% Ethanol.
- Close access door to FACS-BSC.
- Turn off the aerosol containment system after waiting at least 30 minutes.

Liquid Waste:

- All liquid waste within the FACS-BSC must be treated with a final concentration of 10% chlorine bleach and kept in a closed container for 30 minutes before disposal down the sink.
- All precautions to avoid formation of aerosols and splashes of liquid waste must be taken.
- Spills should be treated with 10% chlorine bleach and then with 70% ethanol.

Solid Waste:

- All solid waste (e.g: tubes, pipette tips) within the FACS-BSC must be collected in an autoclavable bag and sealed prior to removal from the FACS-BSC. The bag should then be removed and sealed in a second bag. Operators will then autoclave and dispose of waste into a biohazard red bag.

In the event of accidental exposure to infectious agents:

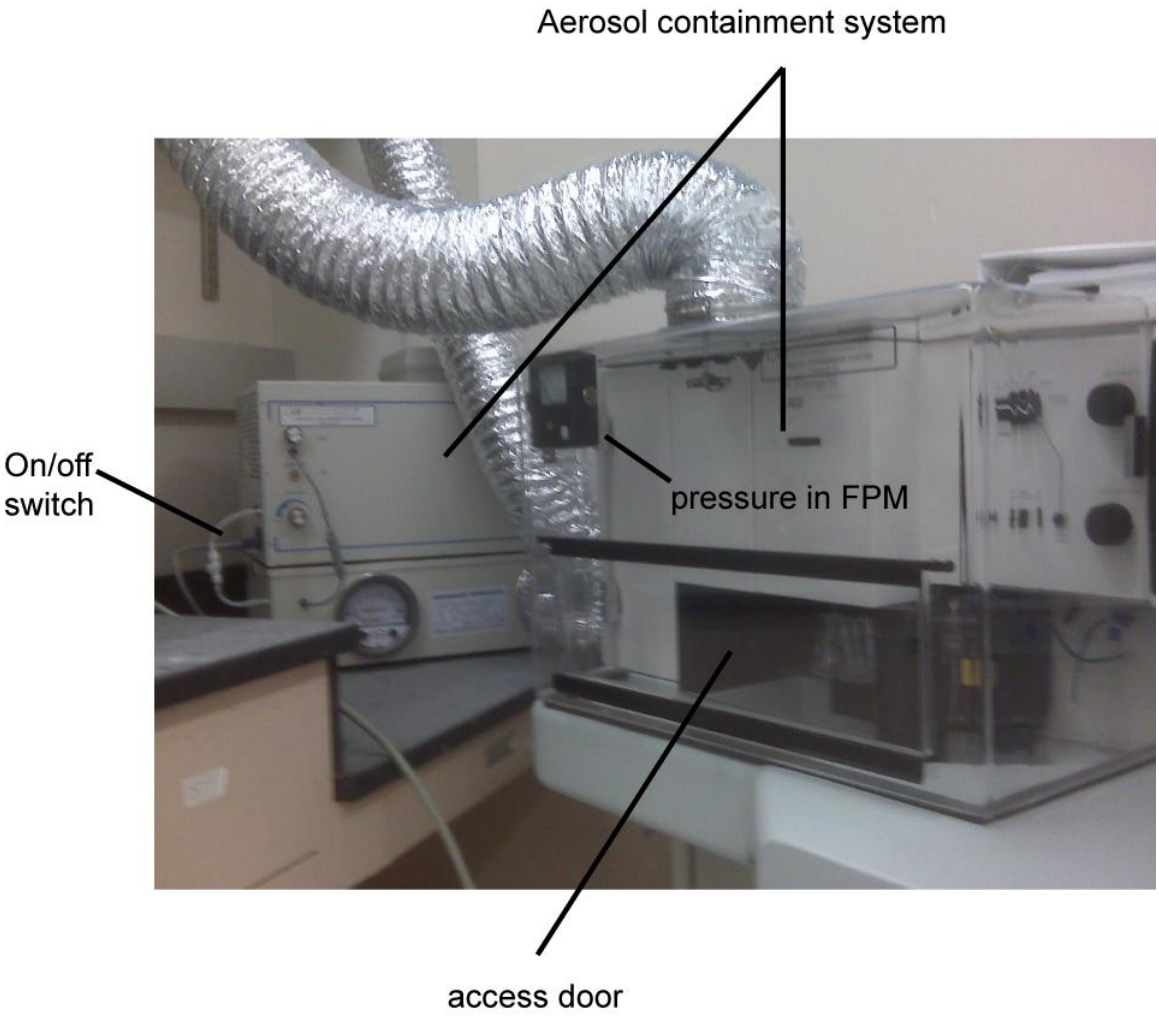
In the event of any malfunction of the FACS sorter (e.g. broken vacuum line(s) or any clogging of the machine), do not try to perform any repairs but inform the person in charge of the sorter [currently Matt Farabaugh, KCC Flow Cytometry (215-503-4556)]. If you notice any signs of failure of the aerosol containment system or any potential leak of the system during the use of infectious material, ask everybody within the laboratory to leave and not to return until future notice. DO NOT TURN OFF THE AEROSOL CONTAINMENT SYSTEM. Immediately inform the Biosafety officer (Sue Gotta, Cell Phone #215-828-2102, or call 811).

After any accidental exposure, go immediately to University Health Services (833 Chestnut Street, Suite 211, phone 215-955-6835) during regular business hours. At all other times, go immediately to the TJU emergency room.

After you are treated, inform your PI about your exposure who will help you to complete a mandatory accident report.

Also, in the event that you develop any illness for which you seek medical treatment, remember to tell your treating physician about any infectious agent to which you may have been exposed in the laboratory. You always need to consider that you might have been infected without your knowledge.

Accidents happen but need to be addressed; failure to follow these procedures may result in dismissal.


Aerosol containment system
On/off switch
pressure in FPM
access door