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Biosafety Principles for Flow Cytometry

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ISAC INTERNATIONAL SOCIETY FOR ADVANCEMENT OF CYTOMETRY

ISAC Biosafety Committee

Mission:

- Establish, update/maintain, disseminate, and promote the use of standards for biosafety principles and procedures related to flow cytometry and cell sorting

Provide guidance on safety related issues including:

- Standard Operating Procedures for sorters and analyzers
- Biosafety aspects of instrument selection
- Risk Assessment procedures
- Unique workflow, procedure, or sample biosafety concerns

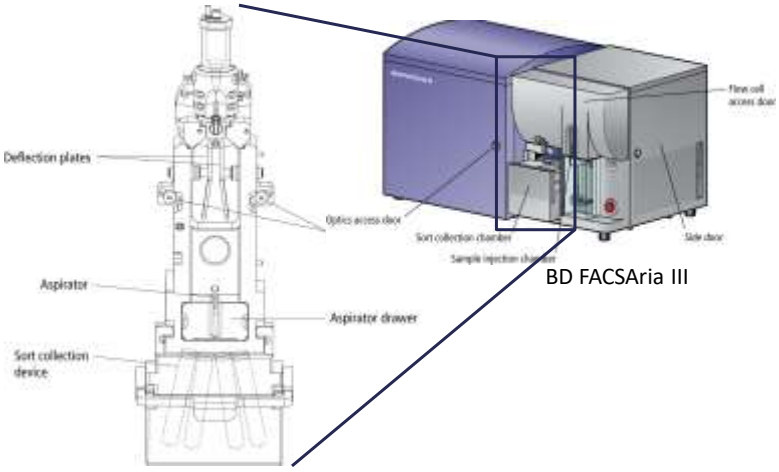
https://isac-net.org/page/Biosafety

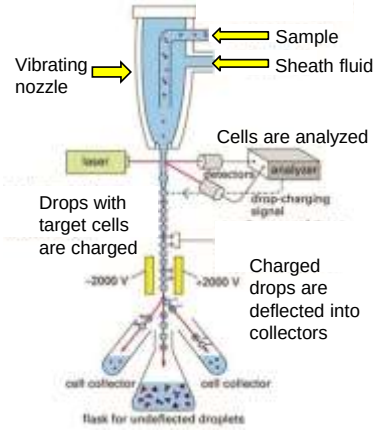
ISAC Biosafety Committee Members:

- Chair: Stephen Perfetto (NIH Vaccine Research Center)
- co-Chair: Kristen M. Reifel (National Biodefense Analysis and Countermeasures Center)
- Avrill Aspland (U of Sydney Centenary Institute, Australia), Jan Baijer (CEA-DSV-IRCM, France), Catherine C. Crumpton (Stanford Stem Cell FACS Core, USA), Iyadh Douagi (NIAID Research Technologies Branch, USA), Benjamin Fontes (Yale Environmental Health & Safety, USA), Evan Jellison (UCONN Health Center, USA), Dominic Jenner (Defence Science & Technology Laboratory, UK), Michael Solga (U of Virginia School of Medicine, USA), Brandon K. Swan (National Biodefense Analysis and Countermeasures Center, USA)

Contact us with your biosafety questions. We're here to help!

Sorting Flow Cytometry





from Molecular Biology of the Cell, 4th edition

3

Biosafety in Flow Cytometry

Several biosafety issues became apparent in the 1990s

- High-speed cell sorters were developed
- Work with unfixed samples was increasing
- Instruments do not fit inside standard biosafety cabinets (BSCs)
- Increased use of shared resource facilities
- No specific mention of flow cytometry in BMBL or other documents

The ISAC formed the Biohazard Working Group

- Published first biosafety guidelines for cell sorting in 1997
- Later became the ISAC Biosafety Committee

The ISAC Biosafety Committee has published several updates to the guidelines

- Holmes et al., 2014, Cytometry Part A 85:434-453
- Reifel et al., 2020, Cytometry Part A 97:674-680

The ISAC Biosafety Committee recommends:

- Locate the cell sorter within a dedicated room with restricted access
- Operate cell sorters within a BSC with an aerosol containment system
- Sort cells at less than 70 psi sheath pressure
- Verify aerosol containment before sorting hazardous material
- Create instrument-specific SOPs that include procedures for nozzle clogs

4

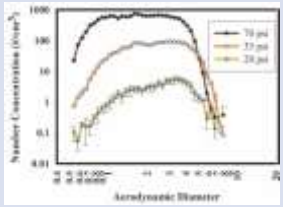
Flow Cytometers Can Produce Aerosols

Study by Holmes et al. to characterize aerosols produced by a sorting flow cytometer and to evaluate aerosol containment methods **(Holmes, KL et al., 2011 Cytometry Part A 79:1000-1008)**

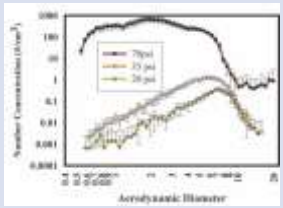
- Testing was done on a jet-in-air sorting flow cytometer (BD FACS Aria II)
- Aerosols were directly measured using a UV APS instrument
- High concentrations of small (**1-5 µm**) aerosol particles are produced during simulated nozzle obstructions at high (**≥70 psi**) sheath pressures
- The aerosol containment system and BSC were successful in containing aerosols, especially at lower sheath pressures

Use of the aerosol containment system was critical to contain aerosols produced during a partial nozzle obstruction

Risk of exposure to aerosols must be mitigated to safely sort infectious/hazardous samples!



Size and concentration of aerosol particles when a partial nozzle obstruction is simulated under different sheath pressures



Size and concentration of aerosol particles when a partial nozzle obstruction is simulated under different sheath pressures, and the flow cytometer is enclosed within a BSC

5

Safety Features of Sorting Flow Cytometers

Features to prevent aerosol generation and/or contain aerosols

- Waste and backflush drains under vacuum
- Automated stream shutoff during a nozzle clog
- Aerosol containment system
- Custom Class II BSC

Features that place barriers or distance between the operator and potential hazards

- Stream viewing cameras
- Physical barrier enclosing the sort compartment
- Physical barrier enclosing the sample station
- Remote viewing of the sort collection area
- Instrument control through software

Other features that prevent exposures

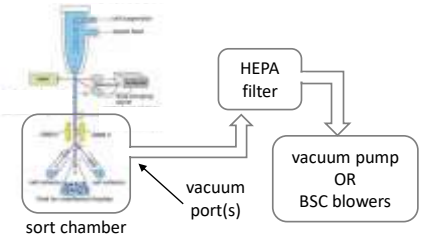
- Continuous fluidics lines
- Decontamination of sample line and waste tank

BD FACSymphony S6 in custom Class II BSC

6

Aerosol Containment System

Aerosol Containment System
aka Aerosol Management System (AMS),
Aerosol Management Option (AMO)



Integrated into a custom BSC

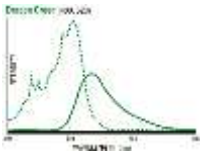
External Buffalo Whisper filter

- A hose or series of hoses attached to opening(s) in the sort collection area that are connected to the blowers of a BSC (for integrated systems) or to an external-filtered vacuum source such as a Buffalo Filter.
- Negative airflow is created inside the sort chamber, and the air is evacuated through the hose(s) into HEPA or ULPA filters within the BSC or the external vacuum source.

Leonard T, 2012. Evolution of Biosafety Guidelines for Flow Cytometry, presentation for MABSA

7

Verifying Aerosol Containment of Cell Sorters

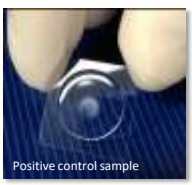


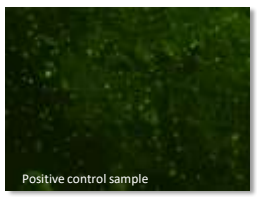
1. Run a sample of small fluorescent beads (1 µm Dragon Green beads)
   run at 70 psi or max working sheath pressure
2. Create an aerosol plume by directing the stream to hit something solid (edge of waste drain)

3. Collect air samples around the sort chamber
   - Aerosol containment system on (TEST samples)
   - Aerosol containment system off (POSITIVE CONTROL samples)

cyclex-d cassette

vacuum pump





4. Count the number of beads present in each sample using a fluorescent microscope
5. If ONE OR MORE beads are seen on the test slides, containment is NOT verified

See Perfetto et al., 2019, Cytometry Part A 95:173-182 and the available video on YouTube and CYTO U.
https://learning.isac-net.org/resources-biosafety

8

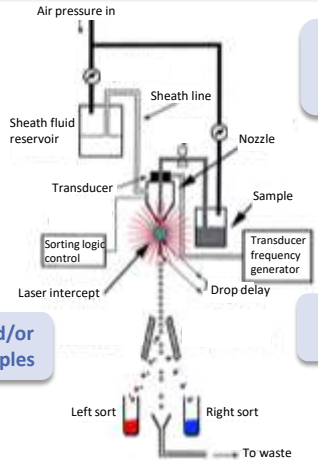
How Do I Assess my Flow Cytometer?

Follow the path of the sample

potential for exposure when accessing components (nozzle, sample line, waste)

potential for aerosol generation if the nozzle clogs

potential for exposure to sample and/or aerosols when retrieving sorted samples



Modified from Michael Ornerod, 3rd Ed, Flow Cytometry, Elsevier, 1999

look for breaks in the sample path where leaks could occur resulting in an exposure

potential for exposure and/or aerosol generation during sample loading/unloading

potential for exposure and/or aerosol generation at waste and backflush drains

potential for exposure at the waste tank

How Do I Assess my Flow Cytometer?

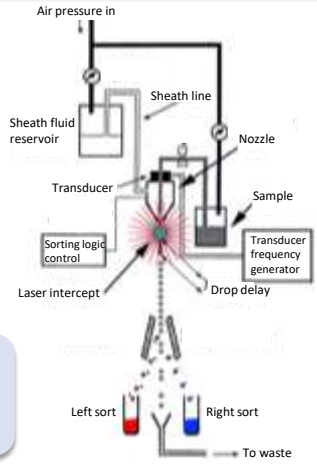
Determine *manufacturer instructions/settings* for safety features

custom Class II BSC or external aerosol containment system

recommended settings of the aerosol containment system

physical barrier at the sort collection area

evacuate aerosols before retrieving sorted samples (run aerosol containment system on high and/or wait a specified time before opening sort chamber)



Modified from Michael Ornerod, 3rd Ed, Flow Cytometry, Elsevier, 1999

available safety features in the software

physical barrier at the sample station

decontamination procedure for sample line and waste tank

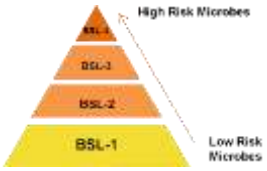
waste drains under vacuum

How Do I Assess my Flow Cytometer?

Perform a *Risk Assessment* to determine safety requirements

Identify the samples/organisms and any hazards associated with those samples

- Microbiological Risk Group/Hazard Group (https://my.absa.org/Riskgroups)
- Pathogen Safety Data Sheets (Public Health Agency of Canada)
- Recombinant organisms, genetic engineering, unknowns



Identify hazards associated with any procedures that will be done with those samples/organisms

- Potential for exposure to samples/organisms
- Potential for generation of aerosols

Determine additional safety measures needed to reduce risk to acceptable levels

- Engineering controls (e.g., BSC, aerosol containment system, labs under negative pressure)
- PPE (e.g., gloves, double gloves, lab coat, safety glasses, shoe covers)
- Respiratory protection (e.g., N95 or PAPR when opening the sort chamber after a sort)
- Aerosol containment testing
- Procedural controls (e.g., maintenance, SOPs, training, approvals and records)

See Holmes et al., 2014 (Cytometry Part A 85:434-453) for details on performing Risk Assessments and assigning a Biosafety Level.

11

How Do I Assess my Flow Cytometer?

Perform an Aerosol Containment Test

Determine a method to generate aerosols from the stream

- Force the stream to impact a solid object
  - Move or tilt the stream to impact the edge of the waste drain
  - Place an object over the waste drain or somewhere that impacts the stream

Perfetto et al., 2003

Identify the recommended settings for safety features and collect TEST samples

- Use the recommended settings to collect TEST sample(s) while generating aerosols
- The TEST sample(s) will determine whether any aerosols escape under a worst case scenario (maximum aerosol generation) when your flow cytometer is functioning normally

Determine the POSITIVE CONTROL setup for your instrument

- Collect POSITIVE CONROL samples with the aerosol containment system OFF
- You may need to prop open doors or barriers to allow aerosols to escape
- You may not see >100 beads in your positive control; determine the appropriate cutoff for your setup
- This sample is intended to show that you created aerosols containing beads, and your air samplers were functioning correctly

Consider wearing RPE when collecting positive control samples!

12

How Do I Assess my Flow Cytometer?

Evaluate regions of concern using the cyclex-d assay

Collect air samples near regions of concern when instrument is functioning normally

- Create an aerosol release using the same method as in the aerosol containment assay
- Use the recommended settings for the instrument and all safety features
- Place cyclex-d air samplers near any areas where aerosols could escape
- Determine the best sampling time depending on desired sensitivity (0.04 aerosols/cm^3 at 10 min collection time)
- This will determine whether aerosols escape under normal operation

Collect air samples with safety features turned off (optional)

- Turn off any safety features you want to test
- Create an aerosol release and collect air samples near areas of concern
- This will determine whether aerosols escape without the use of particular safety features

Incorporate your findings into your Risk Assessment

- Determine whether aerosols are adequately contained using recommended safety features
- Determine whether additional PPE and/or respiratory protection is needed
- Identify any special procedures needed to reduce risk and include in SOPs

13

Don't Forget to Evaluate Your Analyzers

Evaluate other instruments such as analyzers

Several areas of concern were identified on analyzers (Aspland et al., 2021)

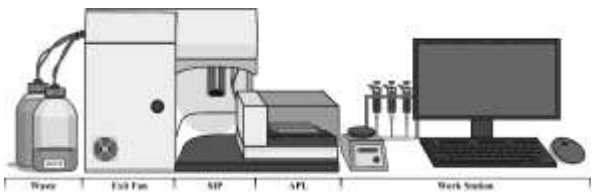
- Sample Injection Port (SIP)
- Exit fans
- Automated plate loader (APL)
- Waste tank

Identify additional areas where exposure to sample or aerosols could occur (evaluate using cyclex-d)

Determine mitigation measures to reduce or eliminate exposure risk

- Additional PPE
- Use of respiratory protection
- Take special care at the SIP (alignment, droplet containment module)
- Use of additional procedural and/or engineering controls (e.g., install filters at all vent holes on the waste tank)
- Incorporate into your Risk Assessment and SOPs

Standard benchtop analyzer workspace with associated equipment



Aspland et al., 2021. Cytometry Part A 99:81-89

14

Example: BD FACSymphony S6

available external Aerosol Management Option (AMO)

sample line, flow cell, nozzle can be decontaminated or replaced

potential for aerosol generation if the nozzle clogs

potential for exposure to sample and/or aerosols when retrieving sorted samples

software control including automated cleaning modes

available custom Class II BSC with integrated Aerosol Management System (AMS)

sample tubes loaded into pressurized sample injection chamber

potential for exposure and/or aerosol generation during sample loading/unloading

potential for exposure at the waste tank

15

Example: BD FACSymphony S6

Prevent exposure to sample

- Prepare samples inside a BSC
- Wear appropriate PPE (gloves, eye protection, etc.)

Prevent exposure to aerosols

- Prepare samples to prevent clogs (free of debris and clumps)
- Run the AMS/AMO while running hazardous samples
- Monitor the sort, and stop sample flow if a clog or other malfunction occurs
- Wear RPE when retrieving sorted samples
- For max protection, place the entire instrument inside a BSC

Properly decontaminate and dispose of waste

- Add disinfectant (e.g. bleach) to the waste tank prior to running samples
- Disinfect sample line/flow cell and any areas exposed to sample after use
- Empty waste after appropriate contact time at least daily

Maintain Equipment

- Complete performance checks and verify aerosol containment before sorting hazardous samples
- Complete annual preventative maintenance and certifications (instrument and BSC)

16

Example: WOLF Microfluidic Flow Cytometer

fits inside a standard Class II BSC

load open tube onto sample station

sorted sample drips into open tubes or microwells

control of streamflow and sorting in software

risk of sample leakage from cartridge

NanoCellect WOLF

operates at very low pressures (2-3 psi)

risk of blockage if fibers are in the sample

risk of blockage with buildup of some samples (e.g. free DNA)

sorting happens within a disposable chip; the stream does not exit through a nozzle

17

Example: WOLF Microfluidic Flow Cytometer

NanoCellect WOLF

Manipulate samples inside a BSC

- Load and unload sample tubes inside a BSC; disinfect tubes and use a secondary container to transport (if operating outside BSC)
- Wear appropriate PPE (gloves, eye protection, etc.)
- Consider wearing respiratory protection if operating the instrument outside a BSC
- For max protection, place entire instrument inside a BSC

Prevent cartridge leaks

- Visually inspect cartridges for defects and DO NOT USE if defective
- Prepare samples to prevent blocks (free of fiber, large clumps, free DNA)
- Monitor the sort, and stop sample flow if blockage occurs

Properly dispose of used cartridges and disinfect any areas exposed to sample

- Cartridges should be disposed in biohazardous waste
- Wipe areas around the sample station and sorted sample collection area with a disinfectant as needed

18

How can you help?

Become part of the team!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Risk Assessments

- Flow cytometry operators are NOT trained to perform Risk Assessments
- They should put together a team involving their institutional safety personnel
- You can help with general laboratory mitigation to reduce sample exposure
- You can verify they are following institutional guidelines

Regulatory compliance

- Identify any local, state, or federal regulations that apply
- Help flow cytometry facilities implement measures to comply with regulations
- Help properly document compliance

Provide risk mitigation measures to reduce exposures

- Help identify potential areas of exposure throughout each workflow
- Recommend risk mitigation measures for other steps: sample transport, common lab procedures (pipetting, centrifuging), disinfection procedures, waste management, etc.

19