Evolution of Biosafety Guidelines for Flow Cytometry: A Change of Sorts

Tom Leonard, PhD
University of Virginia
Flow Cytometry

Medline Publications citing 'Flow Cytometry'

Year

Publications


0 2,000 4,000 6,000 8,000 10,000 12,000

Courtesy: Georgia Health Sciences University Flow Core
Laser Flow Cytometry

Side Fluorescence Light:
RNA/DNA Information

Side Scattered Light:
Internal Cell Structure

Forward Scattered Light:
Cell Size Information

Laser Beam
(\lambda = 633\text{nm})
Pathology Caused by Bioaerosols

- Infection
  (e.g. Tuberculosis, Legionnaire's disease)

- Viability Not Necessary for...

- Hypersensitivity
  (e.g. pneumonitis, rhinitis)

- Toxicoses
  (e.g. neurotoxic symptoms from mycotoxins, febrile illness from endotoxins)
Imperfect Culture

- Viable but not culturable (VBNC)
- Sample collection damages some portion of the organisms.
- Growth medium limitations
- Turnaround = days to weeks.
The Great Plate Count Anomaly

“Typically less than 1% of organisms in the environment can be cultivated by standard enrichment techniques.”

– Norman Pace, ASM News, Excerpt from P&G Award Lecture, American Society for Microbiology.

Fluorescence

- Fluorochromes can bind to a specific cellular component to aid in detection.
  - Metabolic products
  - Monoclonal Ab
  - Nucleic acids
  - Membrane integrity
    - e.g. BacLight
Flow Cytometry Analysis of Viable & Nonviable Aerosolized Bacteria
Flow Cytometry Analysis of Viable and Nonviable Aerosolized Bacteria

Select Publications:
- Chen & Li, J Aerosol Science and Technology, 39:231–237, 2005
Cell Sorting & Aerosols

- Cell sorters purposefully produce droplets
- ...and aerosols.
- Fluid Pressure up to 70psi
NO CLOGGING

These woods are for huntin', fishin' and campin' with great stuff from Bass Pro Shops Outdoor World at Arundel Mills.
Aerosol Production During Nozzle Obstruction

Biohazard Potential

- Unfixed Cells
  - Known pathogens
  - Unknown pathogens
  - Recombinant DNA
  - Considerations
  - Genomic sequences of infectious agents
  - Chemical mutagens
  - Routes of Exposure
1997 International Society for Analytical Cytology (ISAC) Guidelines

- Created out of recognition that sorting was becoming more prevalent, yet hazards of sorting unfixed cells, particularly HIV-infected cells, were not well known.
- Provided awareness of biohazard potential of unfixed cells, sorting hazards, and risk assessment.
- Provided written recommendations for handling and sorting of potentially biohazardous specimens.
1997 ISAC Environmental Controls

- Recommended BSL2 containment combined with BSL3 work practices when sorting unfixed cells.
- Dedicated room for sorting unfixed samples.
- Ideally, “BSL3-type” room
- (-) Pressure & 10ACH
1997 Highlights (cont)

- Mechanisms for Institutional Approval
  - Biosafety Committee
  - Sorting Request Form
- Training & Experience Recommendations
  - ~2 years of FCM experience
  - Experience with potentially biohazardous specimens.
  - Knowledge of characteristics of infectious organism
T4 Bacteriophage on E. Coli Lawns

- Substantial preparation
- Time consuming
- Requires intermediate knowledge of microbiological techniques.
2007 ISAC Update

- Advances in science (e.g. cell biology)
- Advances in safety
- High speed sorting
- New aerosol containment test methods
- LASER safety recommendations
When sorting any infectious or hazardous material, even if it is classified as BSL-2, it is critical to understand that droplet-based sorting procedures are considered BSL-3 practices.

It is therefore recommended that viable, unfixed samples...be sorted at a minimum on a sorter which has been tested for aerosol containment located in an appropriately modified BSL-2 facility using BSL3 practices.
2007 ISAC Standard

- Complete BSL3 containment highly recommended for high speed sorting

- Recommend BSC enclosure for BSL2 sorting.

- Importance of audits to verify containment measures.

- Inspection of fluidics
2007 Validation

- Verification of aerosol management should be performed as often as possible.
- Testing whenever changes are made to sorter that may affect escape of aerosols.
2007: GloGerm Validation
Rapid Turnaround
Active Sampling
Sorter set to 70psi, 50K particles/sec
Fluorescence Microscopy Analysis of Slides
Safety in Flow Cytometry: Selected Highlights

- 1995: ISAC Biohazard Working Group Formed
- 1997: ISAC Biosafety Guidelines published
- 2001: Use of GloGerm as substitute for T4
- 2003: GloGerm modification: Anderson air sampler
- 2007: ISAC Biosafety Standard published
- 2012?: NIH Biosafety Policy for Cell Sorters (in progress)
National Institutes of Health Biosafety Policy for Cell Sorters

- Draft form as of June 2012
- 2009 Task Force to create NIH-wide biosafety policy for cell sorting
- Co-Chairs: Dr. Kevin Holmes & Steve Perfetto
- Intended to apply to intramural NIH research laboratories.
Draft NIH Biosafety Policy for Cell Sorters

- Intended to reduce or eliminate exposure
- Guidance:
  - For the design of laboratories housing cell sorters
  - For the creation of laboratory or instrument specific SOPs
  - On procedures for the safe operation of cell sorters and validation of their aerosol containment systems
# Guidelines for Risk Assessment

<table>
<thead>
<tr>
<th>Risk Assessment Condition</th>
<th>BSL2</th>
<th>BSL2 w/enhanced precautions (during sorting operations)</th>
<th>BSL3</th>
<th>BSL4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Example Sample type or Agents</td>
<td>Uninfected non-primate</td>
<td>Non-infectious Human/NHP cells Infectious but with low risk assessment</td>
<td>Infectious samples with high risk assessment All samples containing known aerosol pathogens</td>
<td>Extremely Dangerous Pathogens</td>
</tr>
<tr>
<td>Containment system validation</td>
<td>Normal murine cells 3rd gen Lentivirus (non-human cells)</td>
<td>Normal human blood Human cell lines Influenza A 2nd gen Lentivirus or 3rd gen in human cells</td>
<td>Mycobacterium tuberculosis Monkeypox</td>
<td>Ebola</td>
</tr>
<tr>
<td>Aerosol containment operating</td>
<td>Periodically (monthly or with filter change)</td>
<td>Periodically (monthly or with filter change)</td>
<td>Prior to each sort</td>
<td>Prior to each sort</td>
</tr>
<tr>
<td>N-95 or better respirator</td>
<td>Required</td>
<td>Required</td>
<td>Required</td>
<td>Required</td>
</tr>
<tr>
<td>PAPR</td>
<td>Optional</td>
<td>Optional</td>
<td>Required</td>
<td>N/A</td>
</tr>
<tr>
<td>Eye Protection</td>
<td>Safety Glasses</td>
<td>Face Shield or safety goggles</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Lab Coat</td>
<td>Front Closure</td>
<td>Wrap Around rear closure</td>
<td>Coveralls</td>
<td>Special suit</td>
</tr>
<tr>
<td>Separate room</td>
<td>Optional</td>
<td>Required or limited access to room</td>
<td>Required</td>
<td>Required</td>
</tr>
</tbody>
</table>

*Courtesy: Dr. Kevin Holmes, NIH*
BSL2 Enhanced Laboratory

- Sorter is to be located in a separate, lockable room where no other lab activity is performed.

- BSC may abrogate requirement for separate room depending on risk assessment.

- Air flow in the room is balanced to create negative airflow into room.

- Visual monitoring device recommended.
BSL2 Enhanced

- Sorting room is locked to:
  - restrict access
  - allow operator to concentrate on sort
  - maintain regular air flow and negative air pressure to room

- Sign placed on lab door to indicate potentially biohazardous sort in progress. Sign should contain all information for entering room safely, including warning for Class IIIb or IV LASERS, if applicable.
BSL2 Enhanced Laboratory Design

- If sorter is located in a shared lab:
  - All PPE requirements apply to all personnel present during sorting.
  - Cell sorter should be placed in a location so that room air flow is toward the sorter and away from other areas of the lab.
Standard Operating Procedures

- Preparation before the sort:
  - Covering control surfaces with plastic wrap, including keyboard and mouse.
  - Sample preparation
  - Check fluids, waste, availability of fresh disinfectant solutions, etc.
  - Containment Validation Testing

- Procedures in the event of a nozzle obstruction, including time to wait prior to opening sort chamber.

- Procedures for Decontamination.
SOPs Continued

- Annual SOP evaluation or when there is a change in instrument configuration.

- Draft Policy offers useful template SOPs for BSL2, BSL2 enhanced and BSL3 SOP for FACS Aria II.
Personal Protective Equipment

- **BSL2**
  - Front closure lab coat, gloves, safety glasses
- **BSL2 enhanced**
  - Isolation style solid-front or wrap around gown and gloves. Goggles or face shield.
    - Respirator use required during sort.
    - Mucous membrane protection for non-primate samples containing agents that do not pose respiratory risk (e.g. leishmania, murine toxoplasma).
Aerosol Management Systems

- All sorters must be equipped with an aerosol management system.
  - Usually consists of an evacuation pump equipped with a HEPA filter before exhausting to the room.
Aerosol Management System Validation

- **BSL2 & BSL2 Enhanced:**
  - Periodically (e.g. monthly or when filters are changed).

- **BSL3**
  - Prior to every sort.
Acknowledgements

- Dr. Kevin Holmes
  Chief, Flow Cytometry Section
  NIAID, NIH

- Joanne Lannigan
  Director, Flow Cytometry Core
  University of Virginia