Biosafety and Cell Sorting: The Essentials

KEVIN L. HOLMES, PH.D.
FLOW CYTOMETRY SECTION, RESEARCH TECHNOLOGIES BRANCH
NIAID, NIH
BETHESDA, MD
Operation of cell sorters in biomedical labs falls under current biosafety standards

- OSHA CFR 1910.1030
- BMBL, 5th edition

But...

Practices, engineering controls, etc. for cell sorters are NOT specifically addressed
Cell Sorter Biosafety Standards

- 1997: ISAC (International Society for the Advancement of Cytometry) Biosafety Guidelines
- 2007: ISAC Biosafety Standard
- 2012: Intramural NIH Biosafety Policy for Cell Sorters
- 2014: ISAC Cell Sorter Biosafety Standards
  - Incorporates NIH Biosafety Policy
  - Emphasis on Risk Assessment and SOP development
NIH Biosafety Policy for Cell Sorters

- Approved in August, 2012
- Intramural NIH Policy
- First specific regulation of cell sorters by the NIH
  - Derived from established biosafety principles
    - BMBL and ISAC Biosafety Standards
- Emphasis on Risk assessment
International Society for the Advancement of Cytometry Cell Sorter Biosafety Standards

Kevin L. Holmes,1* Benjamin Fontes,2 Philip Hogarth,3 Richard Konz,4 Simon Monard,5 Charles H. Pletcher Jr.,6 Robert B. Wadley,7 Ingrid Schmid,8 Stephen P. Perfetto9
ISAC Cell Sorter Biosafety Standards 2014

- Overview of Laboratory Associated Infections (LAI’s)
- Aerosols and cell sorters
- Existing regulatory policies
- Risk assessment as applied to cell sorting
- SOP development/recommendations
- Biosafety Standards for Cell Sorting
Laboratory Associated Infections
Hazards in Cell Sorting Labs

- Laboratory workers have a higher risk of acquiring infections
- No documented case of LAI in cell sorter lab, but...
  1. Cell Sorting is a Laboratory Procedure Hazard
     - Aerosol generation
  2. “General agreement among biosafety professionals... that an aerosol generated by procedures and operations...is the probable source of many LAI’s”
     - BMBL, 5th Ed.
  3. Most LAI’s are unreported
Hazards in Cell Sorting Labs

4. **Infections in laboratory may occur even if not normally transmitted via aerosol in nature**
   - Higher concentrations of organism and Aerosol generating procedures
   - Examples:
     - **Example 1:** Airborne Rabies virus Transmission (JAMA, 1973, 226:1219)
       - 99.8% of cases are from bites of infected animals
       - Lab worker blending rabid goat brains for vaccine studies
       - Worker died 21 days after exposure; Inhalation of aerosolized Rabies virus
     - **Example 2:** 1st documented case of aerosol infection of Scrub Typhus (Orientia Tsutsugamushi) (Infection, 2001, 29:54)
       - Usually transmitted via insect (mite, chigger) route
       - Lab worker disrupted cells on open bench
       - Contracted Scrub Typhus (serotype match)
Aerosols and Cell Sorters
Aerosol Production by Cell Sorters

- Cell sorters produce aerosols
  - ~80-300 µm plus smaller satellite droplets
    - Depends upon nozzle diameter, pressure & ddf
    - Captured by collection tubes and waste drawer
  - ‘...secondary aerosols of various and undefined droplet sizes’ may be produced during failures (nozzle clogs) (ISAC 2007 biosafety standards)
Characterization of aerosols by Cell Sorters: Fail Mode

TSI UV-APS

(Holmes, K.L. Cytometry Part A 2011; 79A, pp. 1000-1008)
Characterization of aerosols by Cell Sorters: Fail Mode

- Maximum of $1.8 \times 10^4$ particles/cm$^3$
- Aerodynamic Diameter of 1 to 5 µm

Higher Pressure = higher aerosol concentration

- Pressures typical of sorter ca 2000’s
- Pressures typical of sorter ca 1990’s
Aerosols and Cell Sorters: Summary

- Sorters can produce high concentrations of aerosols
  - Published study has found that at 70psi, aerosols with concentration of 18000/cm³ can be produced in fail condition.
  - These aerosols are between 1-5µm aerodynamic diameter
- Higher sheath pressure increases concentration and decreases size
- Aerosols in this size range, i.e. 1-3µm:
  - May remain airborne almost indefinitely
  - More likely to deposit in lung alveoli
  - Have been shown to be associated with increased infectivity of some organisms
How to protect the operator from potential exposure

- Containment
  - Cell sorter specific engineering controls
    - Sort Chamber, nozzle area and collection chamber doors
  - Aerosol evacuation
    - Aerosol management systems
  - Sorter contained within biological safety cabinet (BSC)
Risk Assessment as Applied to Cell Sorting
Risk Assessment as Applied to Cell Sorting

1. Identify agent hazards
2. Identify laboratory procedure hazards
3. Make final determination of biosafety level
4. Evaluate proficiencies of staff and integrity of safety equipment
5. Review risk assessment with a biosafety professional
Risk Assessment Step 1: Identify Agent Hazards

- Classification of microbiological agents into Risk Groups (Also known as Hazard Groups (UK))
  - Ability to infect and cause disease
  - Virulence (severity of disease)
  - Availability of preventative measures and effective treatments
- WHO and NIH/CDC criteria: Risk Groups 1-4
  - e.g. Hepatitis B is Risk Group 2; Ebola is Risk Group 4
- Resources:
Risk Assessment Step 1: Identify Hazards: Sample Information Form for Cell Sorting

- Cell Source: human, murine, NHP, etc.
- Sample source: blood bank, Animal facility, outside your Institution, etc.
- Cell type: lymphocyte, cell line, etc.
- Does the sample contain any known infectious agents?
  - List agent and supply IBC approval documentation
  - Any testing performed
- Were the cells genetically engineered, i.e. transduced with gene therapy virus, adenovirus, lentivirus, retrovirus.
  - Describe and provide IBC approval documentation
Risk Assessment Step 2: Procedure Hazards

- Laboratory Procedure hazards and LAI’s
  - 5 routes of laboratory transmission:
    1. Parenteral inoculations: syringe needles/sharps
    2. Spills/splashes on skin or mucous membranes
    3. Ingestion through mouth pipetting
    4. Animal bites & scratches
    5. Inhalation exposure to infectious aerosols
  - 1-4 account for <20% of LAI’s
  - The cause of 82% of LAI’s are unknown, but are presumed to be aerosols
Cell Sorting is a Procedure Hazard

Due to the high probability of aerosol generation

This will affect the final safety level determination

IBC (Institutional Biosafety Committee) recommendation may not be based upon a specific procedure being performed

The NIH IBC asks if ‘aerosol generating procedures are being performed’, such as cell sorting.

If yes, PI is directed to the NIH Cell Sorter Biosafety Policy
Risk Assessment Step 3: Determination of Biosafety Level

- Make final determination of biosafety level
  - select additional precautions as indicated by risk assessment, e.g. PPE requirements
- Biosafety or containment levels
  - NIH/CDC and WHO: BSL1, 2, 3 and 4 (UK: CL1,2,3 and 4).
- Risk Groups correlate with but do not equate to biosafety levels.
  - Transmission in lab may be enhanced over natural setting
  - i.e. aerosol generation
### Biosafety Level Determination for Cell Sorting (2014 ISAC Standards)

<table>
<thead>
<tr>
<th>Risk Assessment Condition</th>
<th>BSL2</th>
<th>BSL-2 with Enhanced Precautions (during sorting operations)</th>
<th>BSL3</th>
<th>BSL4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uninfected non-primate cells</td>
<td>Non-infectious Human / NHP cells</td>
<td>Infectious but with low risk assessment</td>
<td>Infectious samples with high risk assessment</td>
<td>Extremely Dangerous Pathogens</td>
</tr>
<tr>
<td>Normal murine cells</td>
<td>Normal human blood</td>
<td>Human cell lines</td>
<td>All samples containing known aerosol pathogens</td>
<td></td>
</tr>
<tr>
<td>3rd gen Lentivirus (non-human cells)</td>
<td>An example agent is: Influenza A</td>
<td>2nd gen Lentivirus or 3rd gen in human cells</td>
<td>Example agents include:</td>
<td>Example agents include:</td>
</tr>
<tr>
<td>Periodically (monthly or with filter change)</td>
<td>Periodically (monthly or with filter change)</td>
<td>Weekly or before Every Sort</td>
<td>Weekly or before Every Sort</td>
<td></td>
</tr>
<tr>
<td>Required</td>
<td>Required</td>
<td>Required</td>
<td>Required</td>
<td></td>
</tr>
<tr>
<td>Optional</td>
<td>N-95, FFP2 or better</td>
<td>PAPR</td>
<td>Special Suit</td>
<td></td>
</tr>
<tr>
<td>Safety Glasses</td>
<td>Face shield or safety goggles</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Front Closure lab coat</td>
<td>Wrap around, solid-front</td>
<td>Coveralls</td>
<td>Special suit</td>
<td></td>
</tr>
<tr>
<td>Optional</td>
<td>Required or limited access to room</td>
<td>Required</td>
<td>Required</td>
<td></td>
</tr>
</tbody>
</table>

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*a: Example agents are not exhaustive.

*b: Periodically (monthly or with filter change).

*c: N-95, FFP2 or better.

*d: Required or limited access to room.

*e: Required.
**Biosafety Levels for Cell Sorting:**

**BSC’s**

- Majority of sorting will likely be BSL2 or BSL2 w/ enhanced precautions.
- For samples requiring BSL2 w/ enhanced precautions:
  - Sorter placed in BSC is dependent upon risk assessment; not required.
- BSC provides more safety and flexibility of samples that can be sorted.
  - Example: Compare Normal human blood bank donor samples vs. HIV+ samples from Africa.
    - Risk assessment may determine that HIV donor samples require BSC.
- BSC may abrogate need for separate room.
What if my sorter is in a shared lab, and not contained in a BSC?

Can I sort human samples?

Answer:

1. Human samples must be sorted at BSL2 w/enhanced precautions
2. Sorting can be done, but...
   1. During sorting all personnel in lab must wear PPE, door must be closed and signage posted indicating biohazard and PPE requirements.
   2. PPE requirements are in effect for entire sorting procedure.
3. If the sorter is in a Class II BSC, PPE not required for other personnel, but operator **should** wear respirator during sample manipulation.

4. Ideally, sorter should be enclosed within its own room with negative airflow.
Cell Sorters in BSC’s: Summary

1. Need for a BSC is dependent upon risk assessment
2. Sorters cannot just be placed in a BSC: must be certified
   - 2014 Standards: “must be manufactured to meet functional certification criteria for personnel and product protection as defined by NSF 49 (US or CSN EN 12469 (Europe) or JIS K 3800: 2009 (Japan) or AS 2252.2 (Australia).”
3. Can abrogate requirement for separate room for sorter and requirement for PPE (respirators) for all occupants in the shared lab
4. Does not eliminate need for AMS
Step 4: Evaluate Staff Proficiencies and Safety Equipment

- Staff Proficiencies
  - Workers are the 1st line of defense for protecting themselves
  - Deficiencies of worker practices are identified at this step
  - Review of applicable training
Operator training

- Staff proficiency in cell sorter operation and in biosafety procedures
  - Training essential component of cell sorter lab operation
  - Amount of training tied to risk assessment
    - The higher the risk, the more experience/training required
    - Inexperienced operator more likely to circumvent safety procedures
- Review of SOP at regular intervals
  - Review/practice procedures in event of nozzle clog
    - Nozzle clogs: Good news and Bad news
      - Newer sorters may clog less: Data from Aria showed that of 580 samples, there were 17 nozzle clogs (2.9%). Of these, ~75% were unfiltered.
        - Newer sorting technology may clog less often: Good because aerosol production is less probable
        - Newer sorting technology may clog less often: Bad because the operator is less likely to remember the proper procedures in the event of a nozzle clog
  - POST the SOP section for nozzle obstructions PROMINENTLY
Step 4: Evaluate Staff Proficiencies and Safety equipment

- Safety Equipment
  - must be available and functional
    - Aerosol management system on cell sorters (containment testing)
    - Personal Protective Equipment (PPE)
    - If sorter is in a BSC, must be certified
    - Facilities safeguards, such as directional airflow
Containment testing
## Containment Assessment in Cell Sorters

<table>
<thead>
<tr>
<th>Reference</th>
<th>Date</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characterization of Aerosols produced by Cell Sorters and Evaluation of Containment</td>
<td>2011</td>
<td>UV-APS</td>
</tr>
<tr>
<td>Evaluation of Cell Sorting Aerosols and Containment by an Optical Airborne Particle Counter</td>
<td>2015</td>
<td>Optical counter</td>
</tr>
</tbody>
</table>
Containment testing

- ISAC 2014 Standards recommends GloGerm Procedure
- But: new test procedures are being evaluated
- Cyclex D cassettes: non-viable impactor with d50 of 1μm
  - YG beads (Polysciences) or Dragon Green beads (Bangs Labs)
  - New assay measuring phosphate captured on Cyclex (Poster CYTO 2015)

Containment assay Development: Requirements

- Assay can be performed and results obtained on same day
- Sensitive
  - Level of sensitivity/validation
  - Measure aerosol concentration with UV APS simultaneously
  - Compare with expected level of aerosol release with partial AMO failure
- Affordable: not requiring major investment in equipment
- Usable for BSC-enclosed and non-enclosed cell sorters
Standard Operating Procedure Development
SOP (Standard Operating Procedure) Development

- Identify hazards and specify practices to minimize hazards
- Process of writing SOP forces critical evaluation of equipment and procedures
- Is as important as engineering controls!
SOP Development for Sorters: 4 major parts

1. Preparation before the sort
2. PPE requirements
3. Procedures in the event of a nozzle obstruction
4. Decontamination procedures
SOP Development
Instrument Design Considerations

- No commercial sorter has interlock to prevent opening of sort chamber door
- Does the stream shut off automatically when the nozzle clogs?
  - Aria: stream shuts off; Astrios and Influx: stream remains on
  - Ensure that stream is off, or manually turn off
- Are all of the chambers evacuated by AMS?
  - Aria: Sort chamber original design containment, not evacuation
    - Modification (shipped now with Aria)*
    - Requires procedure to evacuate

SOP development: how to approach

- General considerations in 2014 ISAC Standards
- Risk Assessment determined PPE requirements
- Nozzle obstruction with aerosol generation
  - Fail Mode testing to mimic actual conditions
  - Stream must be turned off (automatically or manually)
  - Ensure that sort chamber is evacuated
    - Critically evaluate aerosol evacuation system
    - Increase evacuation rate for at least 2 min
- Decontamination
  - Disinfectant choices, e.g. 1:10 dil. Household bleach (5,250-6,150 ppm Chlorine)
  - Evaluate areas that are potentially contaminated, e.g. sort chamber, sample line, surfaces, keyboard, etc.
Education/References

  - Flow Cytometry Biosafety Course
  - 2013 Tutorial Recordings: “Risk Assessment and SOP Development”
Thank You!