# Cell Sorting Biosafety: Policies and Practices

KEVIN L. HOLMES, PH.D.

CHIEF, FLOW CYTOMETRY SECTION

NIAID, NIH

BETHESDA, MD

# Policies

- Operation of cell sorters in biomedical labs falls under current biosafety standards
  - OSHA CFR 1910.1030 (other International Stand.)
  - BMBL, 5th edition
- But…
- Practices, engineering controls, etc. are NOT specifically addressed

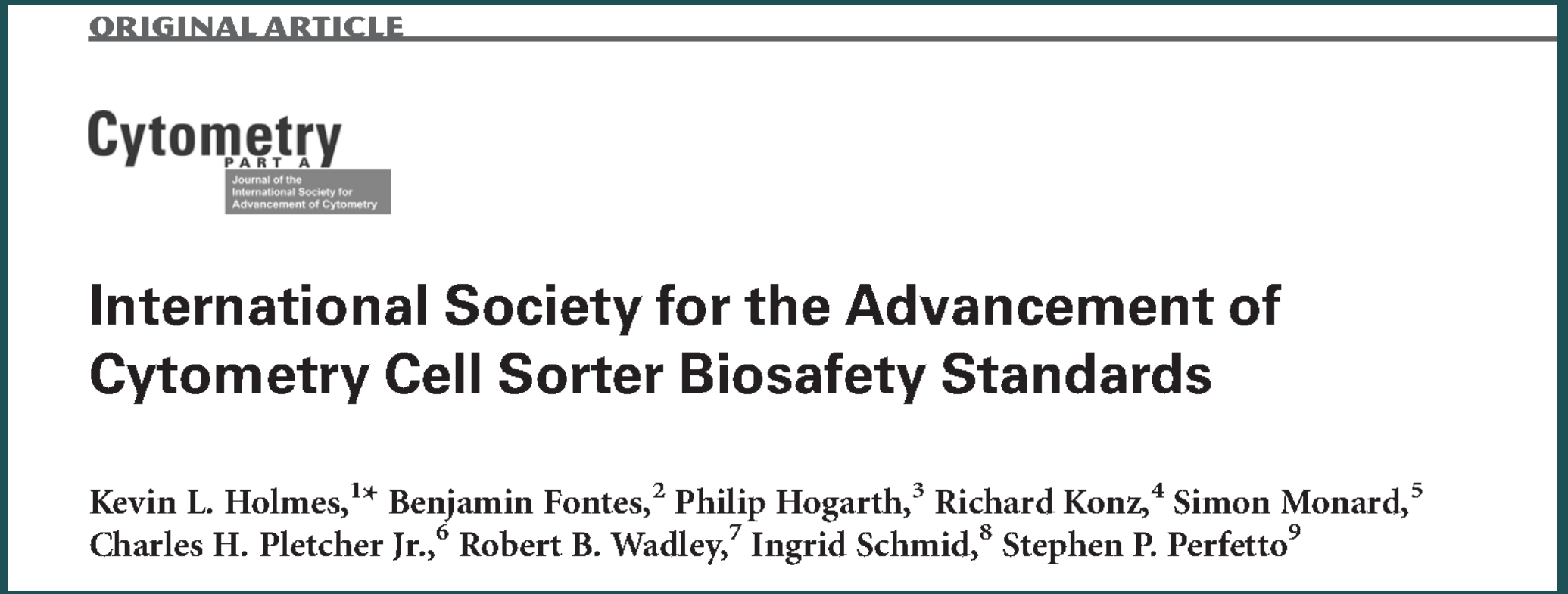
# Cell Sorter Biosafety Standards- History

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

# NIH Biosafety Policy for Cell Sorters

- Task Force formed March, 2009
- 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

# ISAC Cell Sorter Biosafety Standards 2014



ORIGINAL ARTICLE

Cytometry
PART A
Journal of the International Society for Advancement of Cytometry

## 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. Perfetto[9]

# ISAC Cell Sorter Biosafety Standards 2014

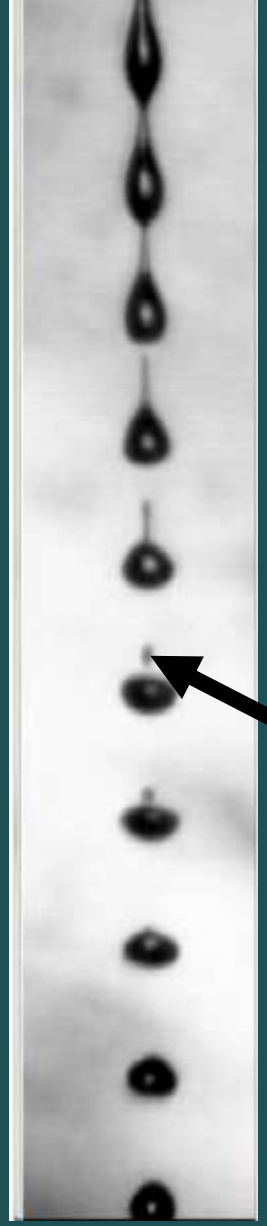
- Overview of LAI's
- Aerosols and cell sorters
- Existing regulatory policies
- Risk assessment as applied to cell sorting
- SOP development/recommendations
- Biosafety Standards for Cell Sorting

# ISAC Cell Sorter Biosafety Standards 2014

- Overview of LAI's
- Aerosols and cell sorters
- Existing regulatory policies
- Risk assessment as applied to cell sorting
- SOP development/recommendations
- Biosafety Standards for Cell Sorting

# 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'produced during failures (clogs) (ISAC 2007 biosafety standards)



# Characterization of aerosols by Cell Sorters: Fail Mode

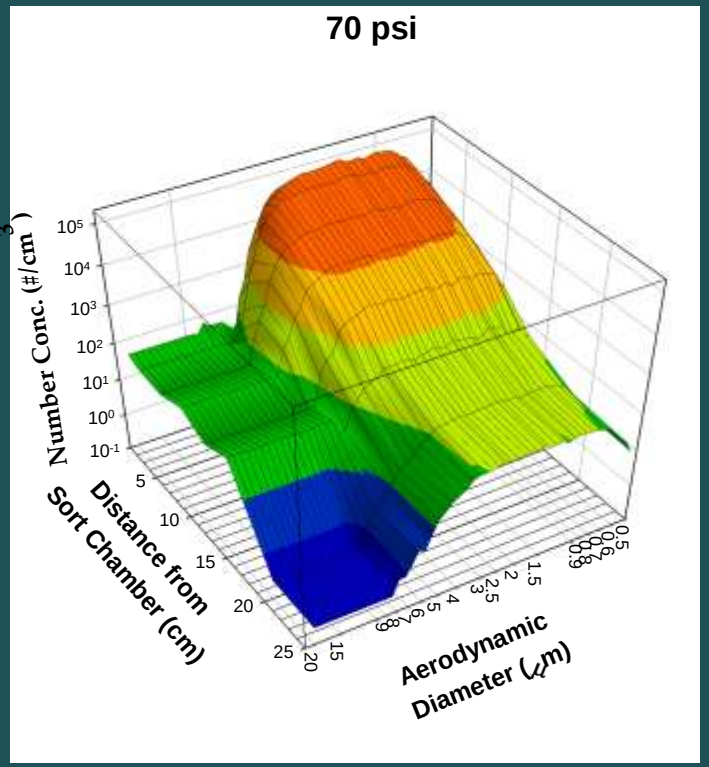
TSI UV-APS


(Holmes, K.L. Cytometry Part A 2011; 79A, pp. 1000-1008)

# Characterization of aerosols by Cell Sorters: Fail Mode

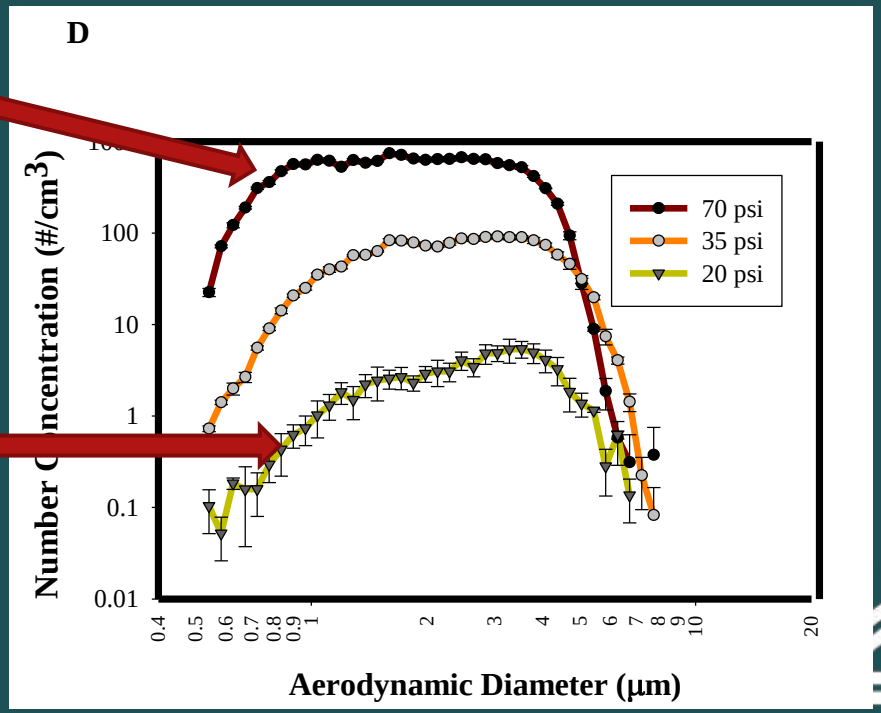
- Maximum of $1.8 \times 10^4$ particles/$cm^3$
- Aerodynamic Diameter of 1 to 5 μm

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
  - At 70psi, aerosols with concentration of 18000/$cm^3$ 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

# Cell Sorter Engineering controls

- Sort Chamber/Collection Chamber doors
- Aerosol Management Systems
- Biological Safety Cabinet

# ISAC Cell Sorter Biosafety Standards 2014

- Overview of LAI's
- Aerosols and cell sorters
- Existing regulatory policies
- Risk assessment as applied to cell sorting
- SOP development/recommendations
- Biosafety Standards for Cell Sorting

# Biosafety Level Determination for Cell Sorting (2014 ISAC Standards)

| | BSL2 | BSL-2 with Enhanced Precautions (during sorting operations) | BSL3 | BSL4 |
|---|---|---|---|---|
| Risk Assessment Condition | Uninfected non-primate cells | Non-infectious Human /NHP cells<br>Infectious but with low risk assessment | Infectious samples with high risk assessment<br>All samples containing known aerosol pathogens | Extremely Dangerous Pathogens |
| Example Sample type or Agents[a] | Normal murine cells<br>3rd gen Lentivirus (non-human cells) | Normal human blood<br>Human cell lines[a]<br>An example agent is: Influenza A[a]<br>2nd gen Lentivirus or 3rd gen in human cells | Example agents include[a]:<br>Mycobacterium Tuberculosis, Monkeypox | Example agents include[a]:<br>Ebola, Marburg |
| Containment System Validated | Periodically (monthly or with filter change)[b] | Periodically<br>(monthly or with filter change)[b] | Weekly or before Every Sort[b] | Weekly or before Every Sort[b] |
| Aerosol Containment Operational | Required | Required | Required | Required |
| Respirator | Optional | N-95, FFP2 or better[c] | PAPR | Special Suit |
| Eye protection | Safety Glasses | Face shield or safety goggles | N/A | N/A |
| Lab Coat | Front Closure lab coat | Wrap around, solid-front | Coveralls | Special suit |
| Separate Room and Environmental controls | Optional | Required or limited access to room[d] | Required[e] | Required[e] |

# Risk Assessment: Some Examples

- Disclaimer:

The final risk management SOP should be selected based on risk assessment and endorsed by the cell sorting facility manager, biosafety professionals and the IBC.*

*ISAC Cell Sorter Biosafety Standards: page 446

# Sorting human cell lines

- *"Do we really need to treat the sorting of human cells lines like 293T, Hela, BJAB, U937, K562, KG1a cells as human primary samples?"*
- Answer: Yes, Human cell lines are sorted at BSL2 w/enhanced precautions
- Background:
  - Letter from OSHA to ABSA president: interpretation of BPS (29 CFR 1910.1030) for human cell lines (1994)
  - "Established human cell lines * which are characterized * * to be free of contamination from human hepatitis viruses, human immunodeficiency viruses, and other recognized bloodborne pathogens, are not considered to be OPIM and are not covered by BPS…All primary human cell explants from tissues and subsequent in vitro passages…handled with the BPS."
  - OSHA leaves it to interpretation by local biosafety office

# Sorting human cell lines

- Ask the Experts-Biosafety Requirements for Human Cell Lines (Keene, J.H., Applied Biosafety: 8, No.2, 2003)
  - However, "it is impossible to prove the negative" (Jack Keene, ABSA)
  - What about unknown contamination?
- ATCC: "It is strongly recommended that all human and other primate cell lines be handled at the same biosafety level as a cell line known to carry HIV or hepatitis virus"
- NIH Biosafety Policy for Cell Sorters: Treat human lines as OPIM due to NIH experience with supposedly 'clean' cells being infected with HIV AND because of aerosol hazard
- NIH Exposure Control Plan: "Regarding cell lines, because it is not possible to test every cell line for every possible virus or ever make the claim that any particular cell line is pathogen free, **we recommend that all human cell lines be accorded the same level of biosafety consideration as a line known to carry HIV.**"

# Lentiviral vectors

- An investigator wishes to sort the following:
  - Primary human glioma cells that have been transduced with a lentiviral vector.
    - The lentivirus has been packaged using Life Technologies' ViraPower Packaging Mix
  - Under what biosafety/containment level do you sort these cells?

A. BSL2
B. BSL2 w/enhanced precautions
C. BSL3

# Lentiviral vector

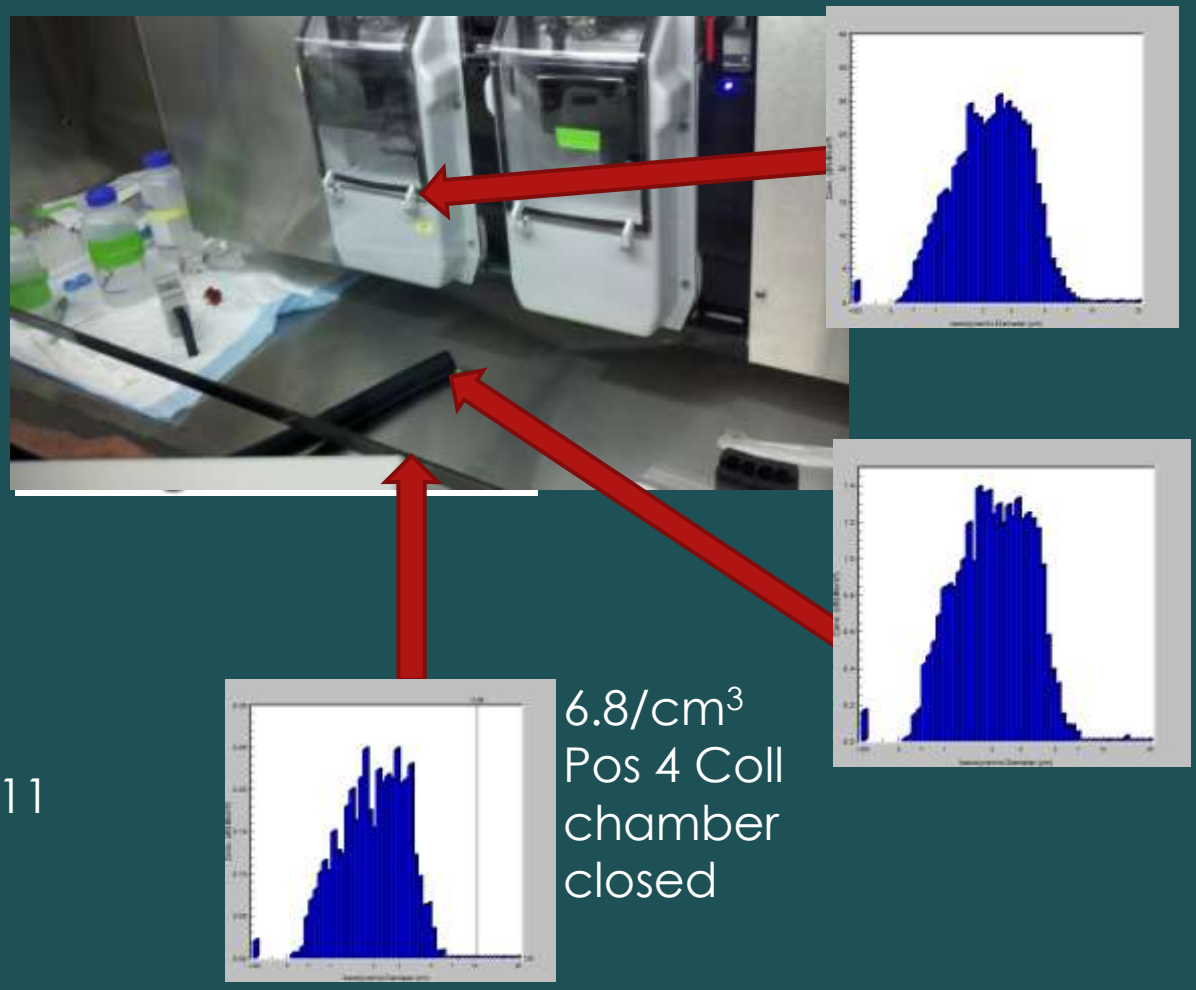
- Answer: BSL2 w/enhanced precautions
- Explanation:
  - The lentivirus is a 3rd generation system, and can be safely sorted under BSL2.*
  - However, the transduced cells are primary human cells and require BSL2 w/enhanced precautions

*All experiments involving recombinant DNA must be approved by the Institutional Biosafety Committee (or equivalent as each country implements directive 98/81/EC.) IBC or equivalent will determine whether lentiviral packaging is 2nd or 3rd generation.

# Cell Sorters in BSC's

1. Need for a BSC is dependent upon risk assessment
2. Sorters cannot just be placed in a BSC: must be certified
    1. 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

# Why you need an AMS inside a BSC




Conditions:
BSC: On
Fail mode
Measurement locations as shown using
UV-APS particle sizer

Data from I-Cyt Reflection,
Poster, J. Lannigan & K. Holmes, ISAC 2011

# Sorter in a BSC-AMS requirement

- Rationale for AMS requirement
  - It's about containment at the source!
  - Class II BSC's are partial containment devices
  - Hand movement can cause air to be expelled
  - AMS contains aerosols within sorter, BSC becomes secondary containment
- Must have an AMS, "in which aerosol containment validation can be performed independent of the BSC blowers"*
  - "This is done to provide greater sensitivity when performing the cell sorter AMS containment tests."*

*ISAC Cell Sorter Biosafety Standards: page 445

# Cell Sorter in a Shared Lab

- *"My sorter is in a lab with other analyzers, and the Investigator wants me to sort human PBMC's from blood bank donors."*
- Answer:
  1. Human samples must be sorted at BSL2 w/enhanced precautions
  2. During sorting: All personnel in lab must wear PPE, door must be closed and signage for posted indicating biohazard and PPE requirements.

# Cell Sorter in a Shared Lab continued

- Ideally, sorter should be enclosed within it's own room with negative airflow
- If the sorter is in a BSC, PPE not required for other personnel, but operator should wear respirator during sample manipulation

# ISAC Cell Sorter Biosafety Standards 2014

- Overview of LAI's
- Aerosols and cell sorters
- Existing regulatory policies
- Risk assessment as applied to cell sorting
- SOP development/recommendations
- Biosafety Standards for Cell Sorting

# 3. SOP (**S**tandard **O**perating **P**rocedure) Development

- Identify hazards and specify practices to minimize hazards
- Process of writing SOP forces critical evaluation of equipment and procedures

# 3. 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: 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
    - Modification (shipped now with Aria)*
    - Requires procedure to evacuate

*(Holmes, K.L. Cytometry Part A 2011; 79A, pp. 1000-1008)

# Containment Testing of the AMS

- Why do it
- When to do it
- How to do it.

# Containment testing: why?

- Any system can fail
- Instrument manufacturers generally make no claims about efficacy of containment of the Aerosol Management System
- Visual tests like smoke tests are unreliable for aerosols in this size range

# Containment testing: When

Based upon Risk Assessment, but must be performed:*

1. Following instrument service or maintenance involving the sort chamber and/or AMS hose connections.
2. Following initial instrument installation or relocation.
3. Following change out of the standalone AMS filter.
4. For BSL3/4 labs:
    1. Prior to every sort if the frequency of sorting is once/week or less
    2. Weekly, if the frequency of sorting is multiple sorts/week

*ISAC Cell Sorter Biosafety Standards: page 445

# Containment testing: How

- GloGerm assay – Published standard*
- Cyclex d assay using polysciences beads
- Future- beadless?

*See: http://isac-net.org/Resources-for-Cytometrists/Biosafety.aspx for ISAC 2014 Standards

# Education/References

- 2014 ISAC Cell Sorter Biosafety Standards (Cytometry Part A, 85A: 434-453, 2014.) Available on ISAC web site:

(http://isac-net.org/Resources-for-Cytometrists/Biosafety.aspx)

- ISAC CYTO U: (http://cytou.peachnewmedia.com/store/provider/provider09.php)
  - Flow Cytometry Biosafety Course
  - 2013 Tutorial Recordings: "Risk Assessment and SOP Development"

# Acknowledgements


- ISAC
  - Biosafety Committee, Ingrid Schmidt, Steve Perfetto
- Biosafety Professionals
  - Jeff Potts, NIH
  - Debbie Wilson, NIH