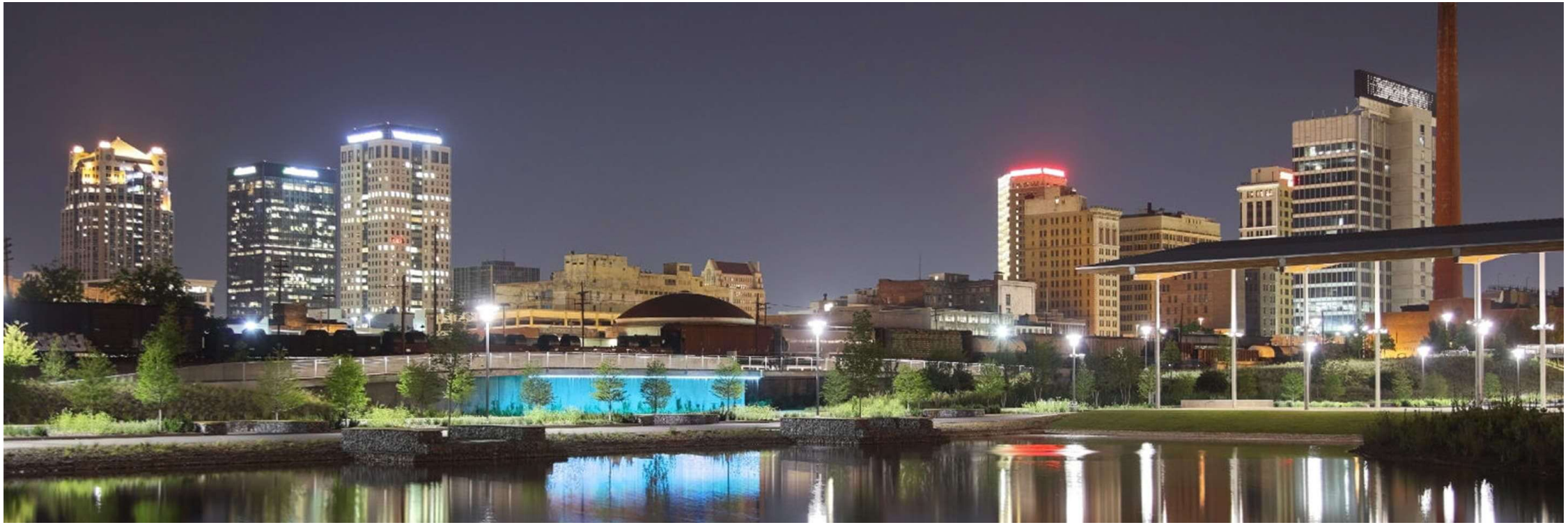
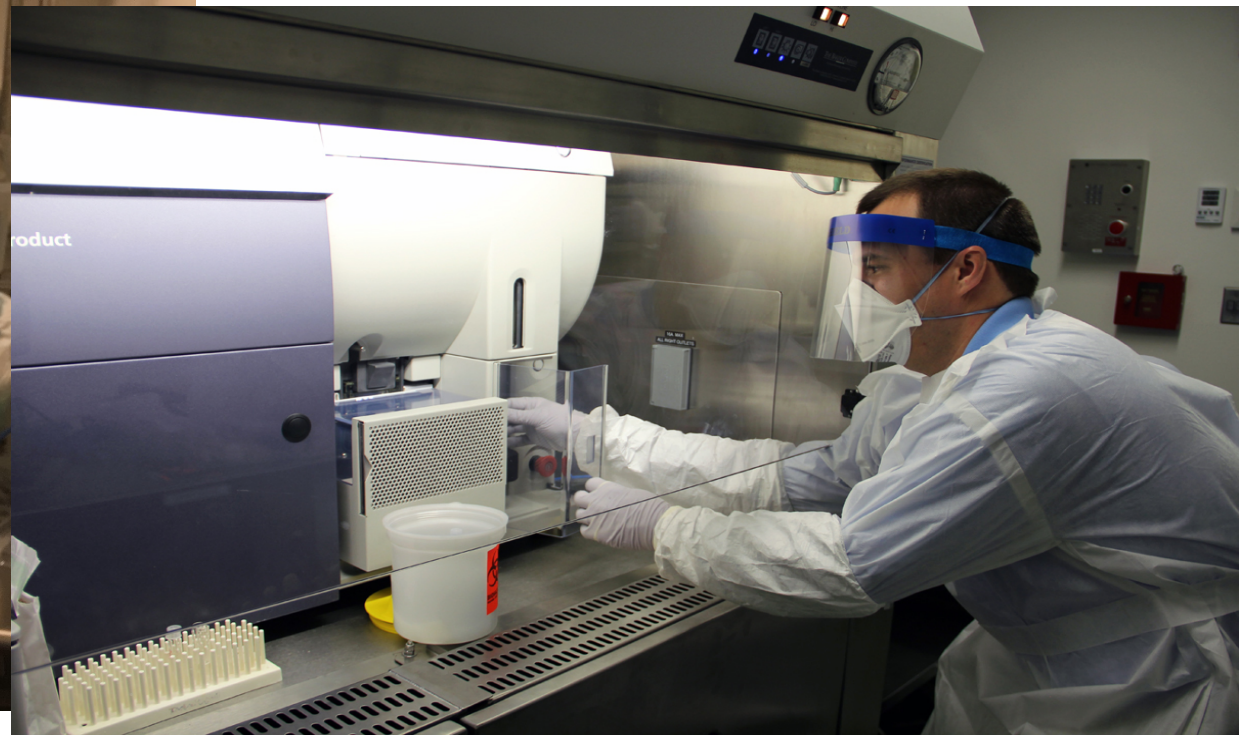
Novel Validation Method for Evaluating Biocontainment of Cell Sorting Flow Cytometers



Benjamin Fontes, CBSP, MPH (For Geoffrey Lyon, MPH)
Yale University Environmental Health and Safety
ABSA International Conference November 20, 2019 Birmingham, AL

Geoffrey Lyon, MPH (at work!)
ISAC Biosafety Committee Member



ISAC Cell Sorter Biosafety Standards 2014

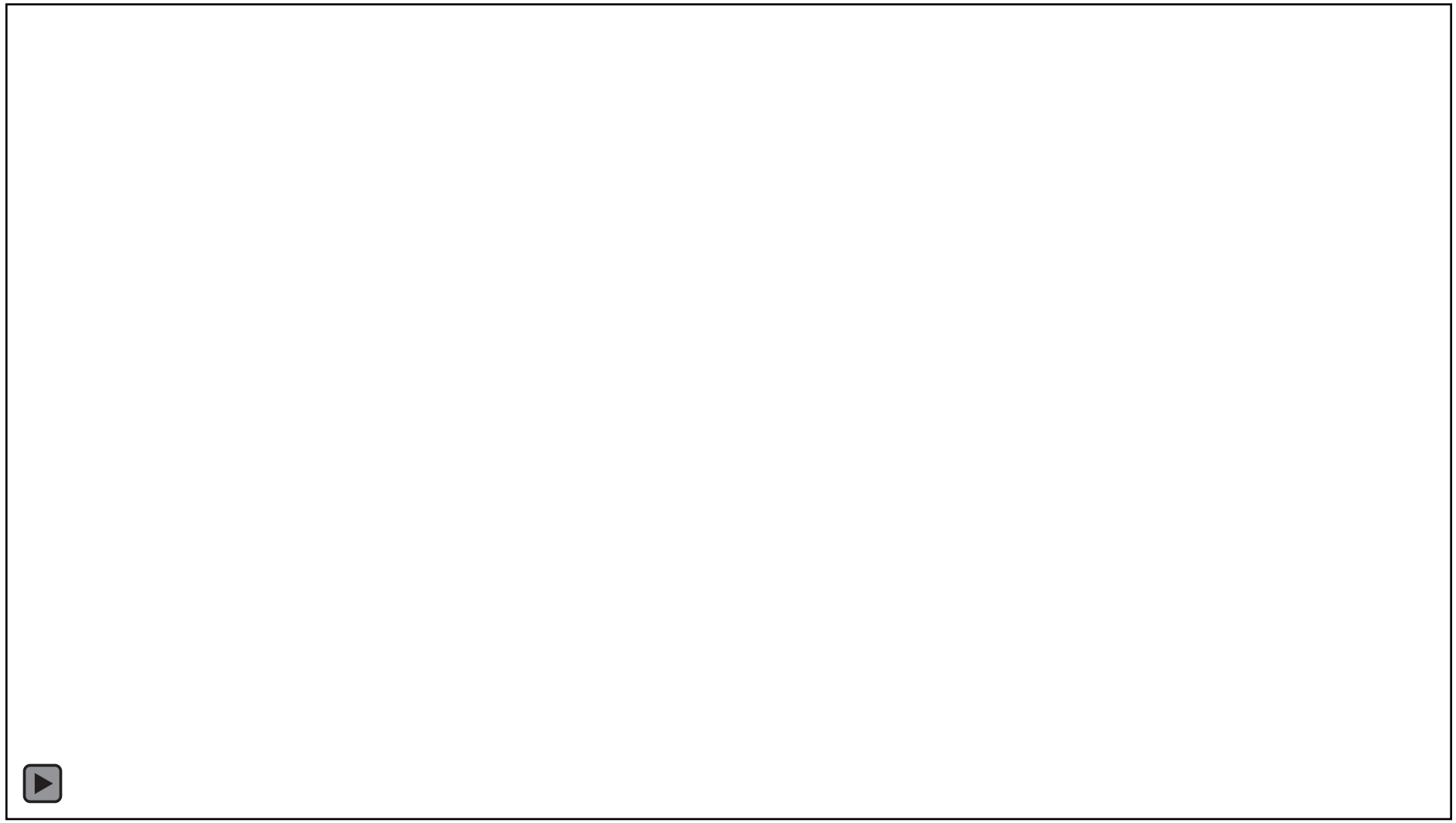
ORIGINAL ARTICLE

Cytometry Part A • 85A: 434–453, 2014

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]



Latest Published Standard for AMS and BSC Containment Testing

Cytometry PART A
ISAC Journal of Quantitative Cell Science

MIFlowCyt

Novel Impactor and Microsphere-Based Assay Used to Measure Containment of Aerosols Generated in a Flow Cytometer Cell Sorter

Stephen P. Perfetto,[1*] Phillip J. Hogarth,[2] Simon Monard,[3] Ben Fontes,[4] Kristen M. Reifel,[5] Brandon K. Swan,[5] Jan Baijer,[6] Evan R. Jellison,[7] Geoffrey Lyon,[8] Patty Lovelace,[9] Richard Nguyen,[1] David Ambrozak,[1] Kevin L. Holmes[10]

Cytometry Part A • 95A: 173–182, 2019

Testing Objectives –

1) Testing must be performed with results in the same day
   - Easily completed in 30-minutes ✓

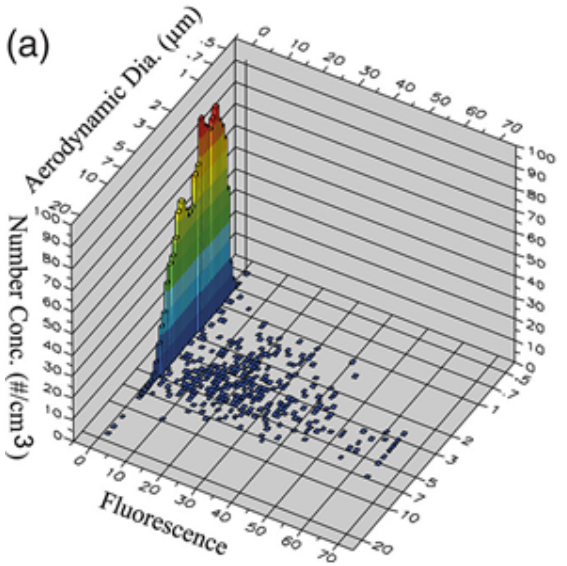
2) Sensitivity must be high and validated via other testing methods
   - 0.04 aerosols/cm^3 sensitivity level validated via AMS restriction and UV-APS ✓

3) Equipment and supplies must be affordable and commonly available to flow labs
   - Cyclex-D cassettes ~$5/cassette, pump and rotameter ~$200, Dragon Green beads ~$175 (many labs will have them for reference purposes already) ✓

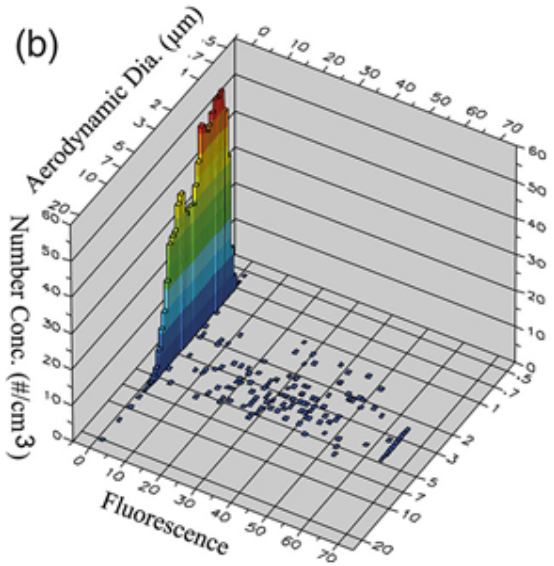
Novel Impactor and Microsphere-Based Assay Used to Measure Containment of Aerosols Generated un a Flow Cytometer Cell Sorter, Perfetto (2019)

UV-APS Testing-
(Ultra-violet aerodynamic particle sizer)

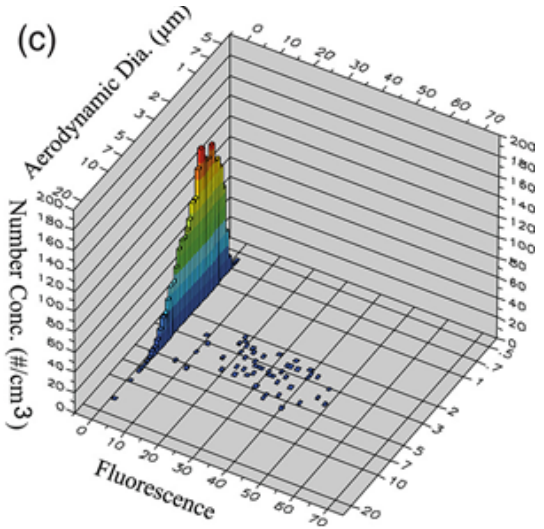
- Pletcher's Cyclex-D test used 0.5-20 µm particles
- Sorters produce high concentration of 1-3 µm aerosols
- 0.75 µm YG beads contained largest range of AD aerosols
- **1.0 YG beads very similar to 0.75 beads and easier to detect**



0.75um YG



1.0um YG



2.0um YG

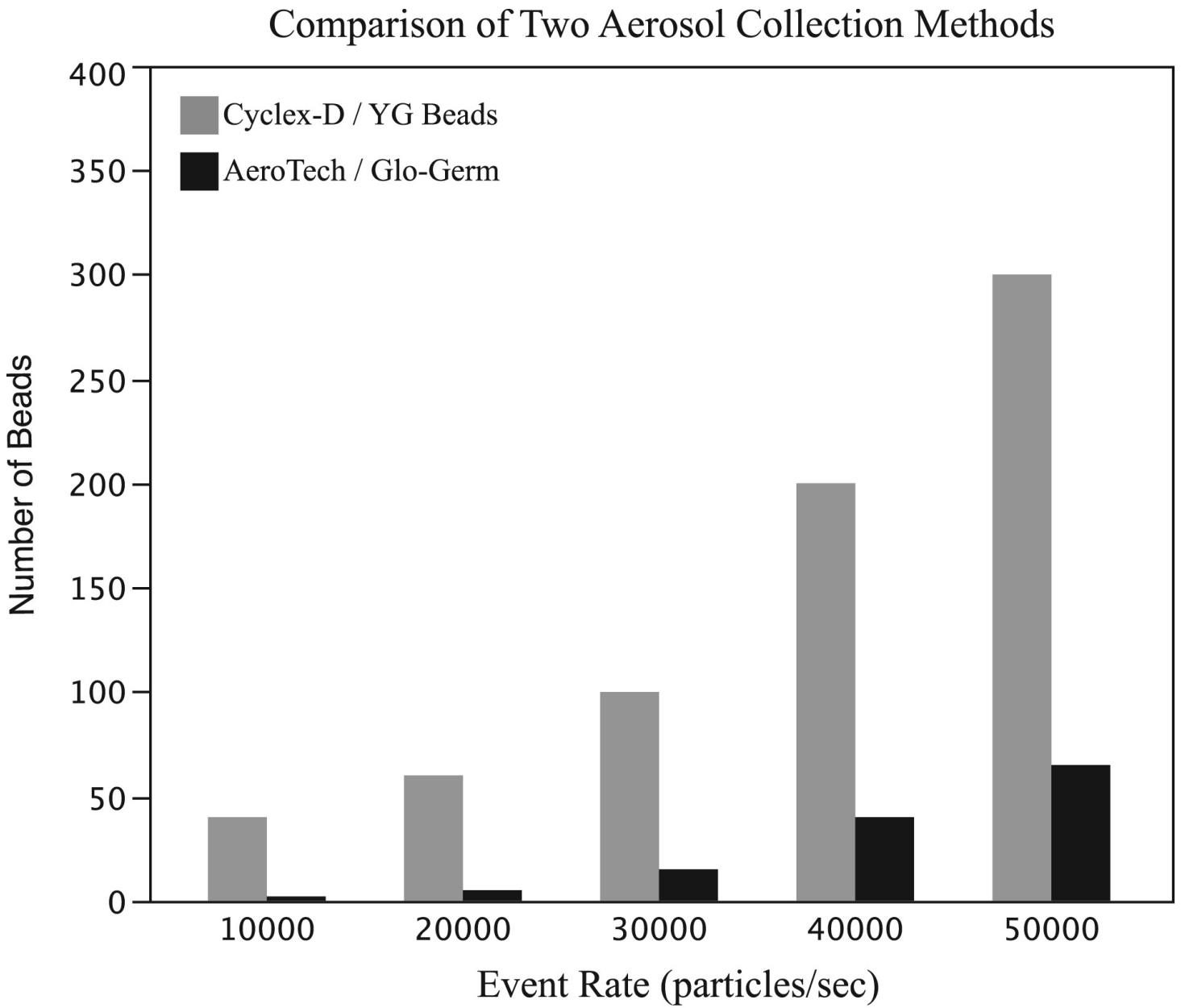
Testing Objectives –

4) **Accuracy must be high with few false positives for enclosed or non-enclosed sorters**

- Test is easily adapted to enclosed or non-enclosed sorters with no problems with ambient aerosols invalidating test results ✓

5) **Efficiency of collection of aerosols in the AD range produced by modern sorters must be high**

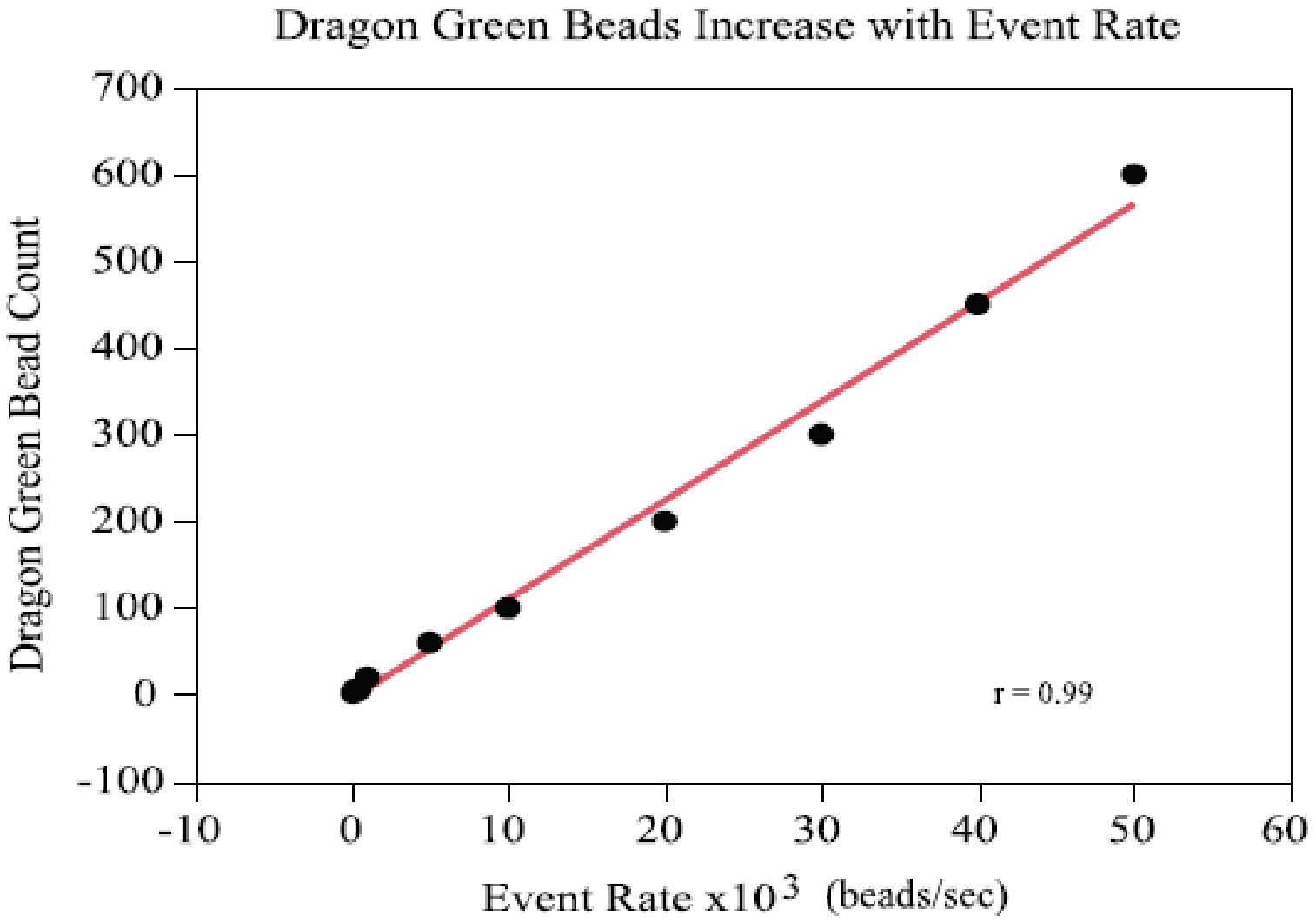
- UV-APS validated that 1.0 µm beads occupied aerosols within the 1-3 µm and were easily identifiable under low magnification, Cyclex-D and DG beads 3x more efficient than Aerotech and Glo-Germ beads ✓



Novel Impactor and Microsphere-Based Assay Used to Measure Containment of Aerosols Generated un a Flow Cytometer Cell Sorter, Perfetto (2019)

Cyclex-D Dragon Green Beads Failure Mode Test-

- Dragon Green beads followed a linear relationship



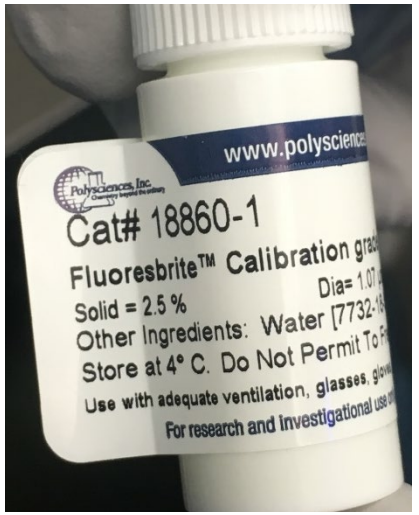
Novel Impactor and Microsphere-Based Assay Used to Measure Containment of Aerosols Generated un a Flow Cytometer Cell Sorter, Perfetto (2019)

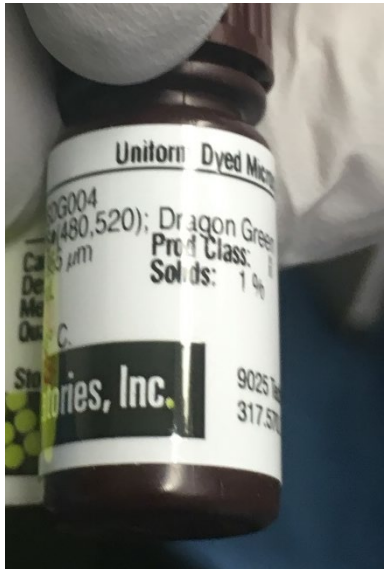
Evolution of Testing Methods-

Author(s)	Test Material	Cost	Time	Active	Other
Merrill (1981)	T-4 Phage (Passive)	$$		Passive	Requires extra equipment, reproducibility issues
Oberyszyn, Robertson (2001)	Glo-Germ (Passive)	$		Passive	Introduction of non-phage containment test
Perfetto (2003)	Glo-Germ (Active)	$$		Active	Bead-based assay utilizing Aerotech impactor
Pletcher (2004)	YG Beads (Active)	$		Active	Convenient bead-based assay, disposable impactor
Holmes (2011)	Aerosols (Real-time)	$$$$		Active	Excessive cost for some labs
Perfetto (2019)	DG Beads (Active)	$		Active	Quick results, high sensitivity, low background, affordable

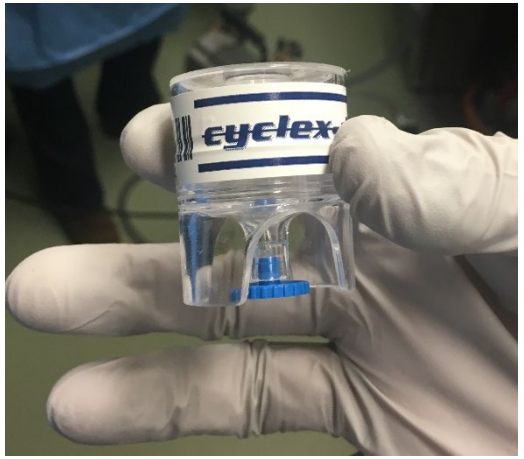
1 **Dragon Beads (DG) favored over Yellow Green (YG) Beads because they are: a) hydrophylic and thus easier to clean; and b) are brighter under the fluorescent microscope and slightly easier to see.**

2019 Update of ISAC Cell Sorting Biosafety Standards



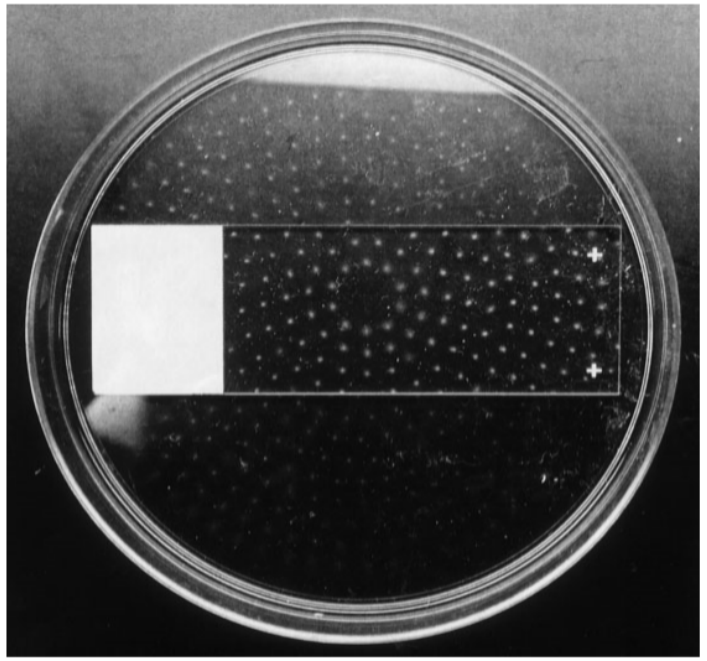


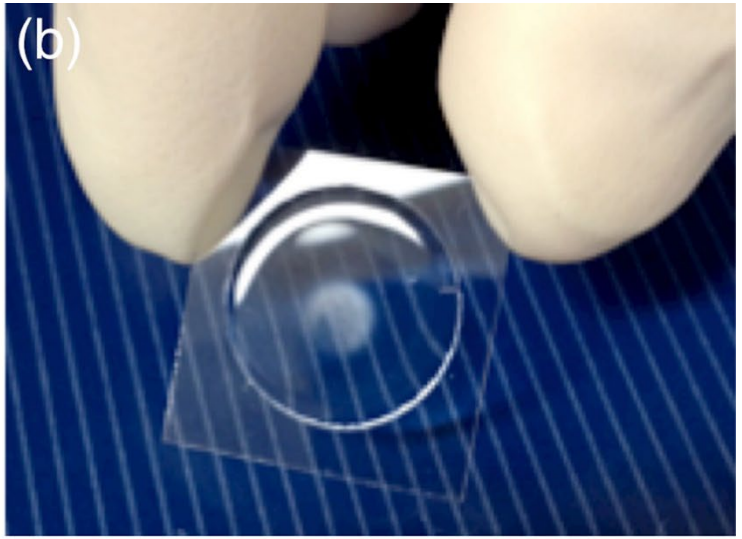
- **Testing simplified**
  - Use of **uniform fluorescent beads** in place of Glo-Germ solution (2014 ISAC Standard)
    - Charles “Hank” Pletcher Jr. & Geoff Lyon
      - Easier to identify when reading slides
      - Known and repeatable bead size
      - Easier to clean the sorter following testing
  - **Disposable** Cyclex-D sampling cassette (in place of aerosol impactor)
    - Simplified collection method & **adhesive** bead collection surface
    - Eliminates clean up between tests
  - **Fewer tests** (down to 2 from 4: positive and negative only)
  - **10 minute** sampling time period (formerly 5 minutes)



Original Glo-Germ Revisited

- **Inconsistent flow rates**
- **Non-intended use**
- **Non-uniform droplet size**
- **Sampling Vacuum considerations**
- **Cleaning**



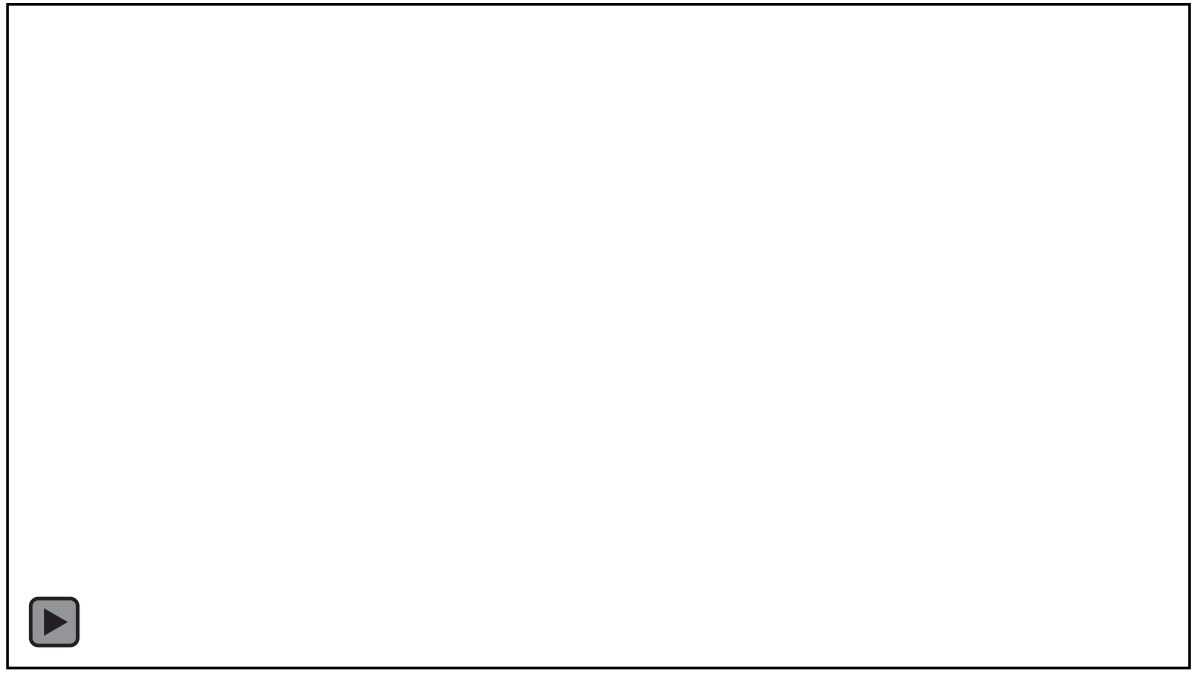


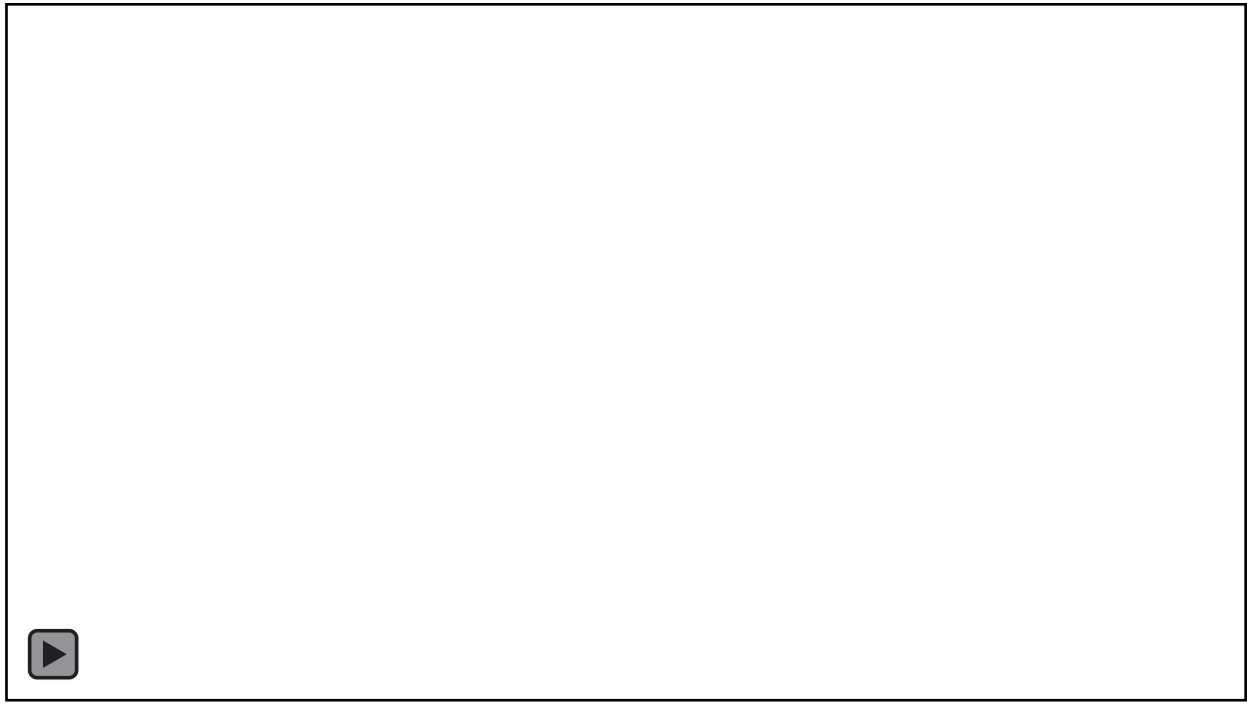
https://healthybuildingscience.com/2013/02/14/mold-testing-air-quality/*

Novel Impactor and Microsphere-Based Assay Used to Measure Containment of Aerosols Generated un a Flow Cytometer Cell Sorter, Perfetto (2019)

Why Test?

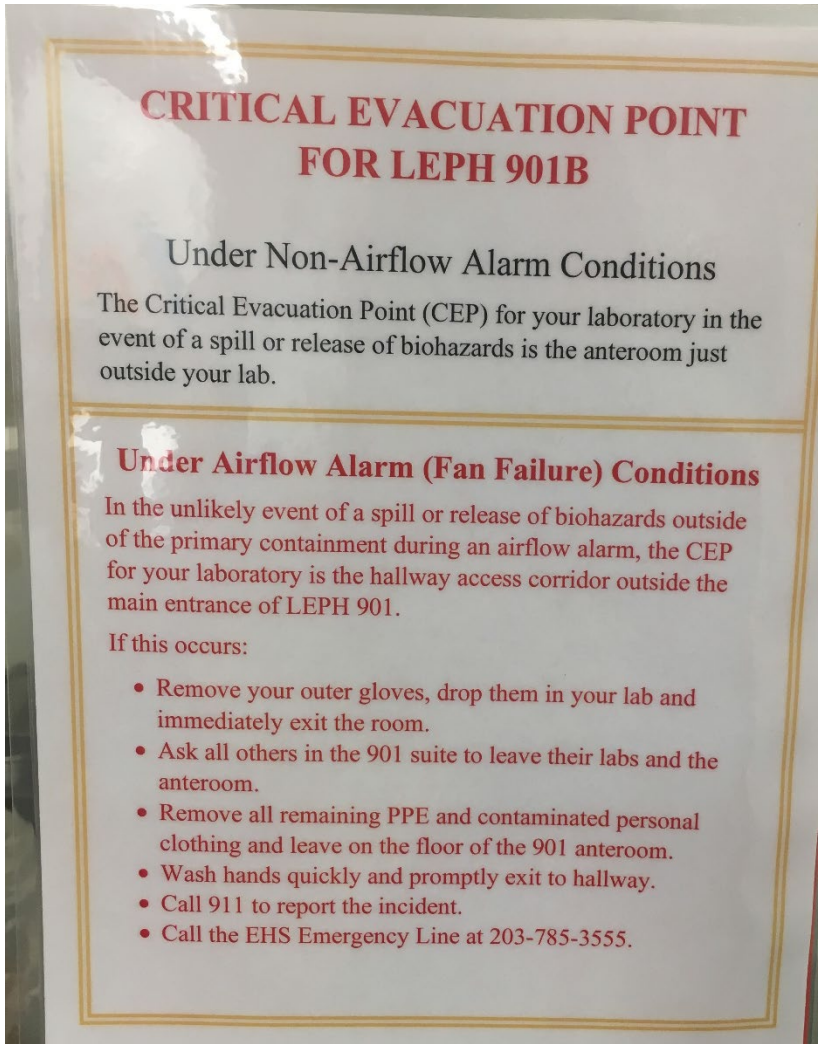
- Up to 4% of sorts result in clogs or deflections like the video
- Inform your risk assessment and risk management
- TO VERIFY YOUR CONTAINMENT
- To identify where aerosols will go during a clog or a deflection



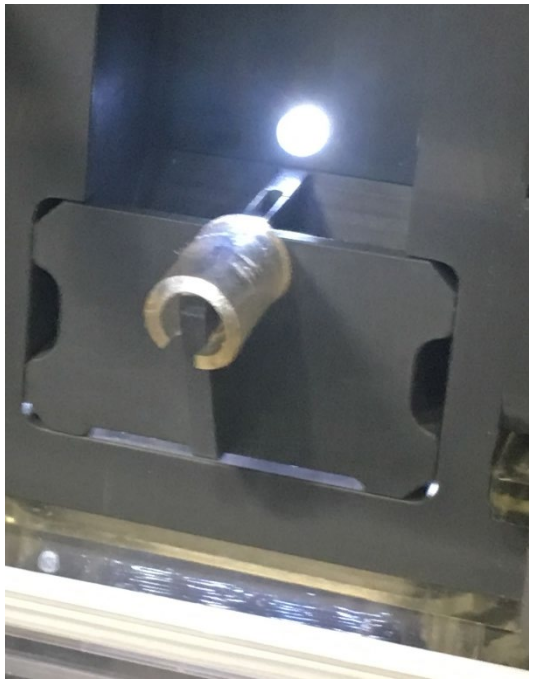


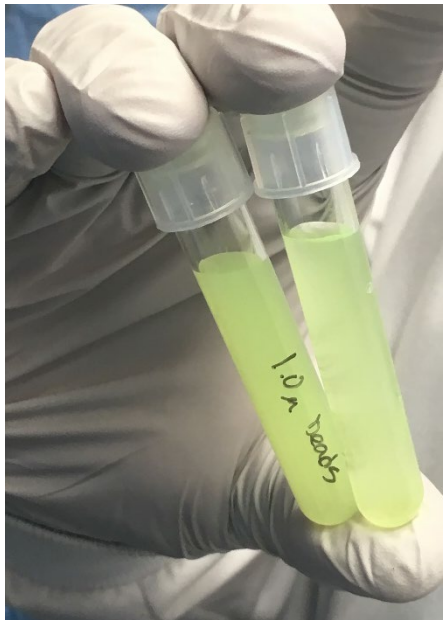
Why Test?

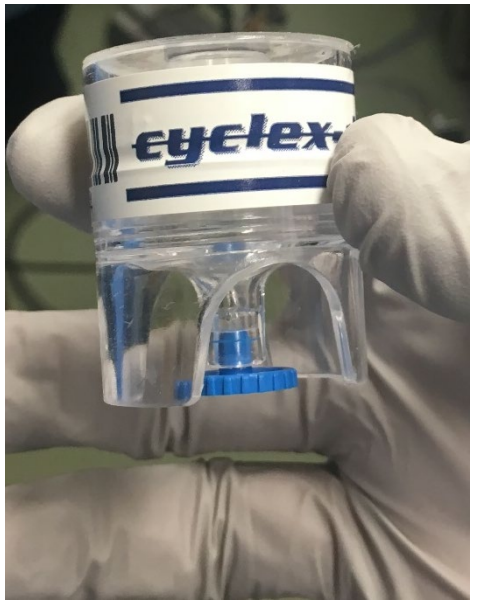
- To aid in your standard operating procedures
  - What requires decontamination after a clog or deflection
    - Inside and outside of the sorter
    - Inside of the enclosure (the room?)
- For emergency response protocols
  - Do you have a **Sp**ill **o**r **Re**lease **O**utside **O**f **P**rimary **C**ontainment?
  - A **SpoRe-OOP-C**!
  - Are occupants trained to evacuate immediately?
  - What is the Critical Evacuation Point (CEP)?

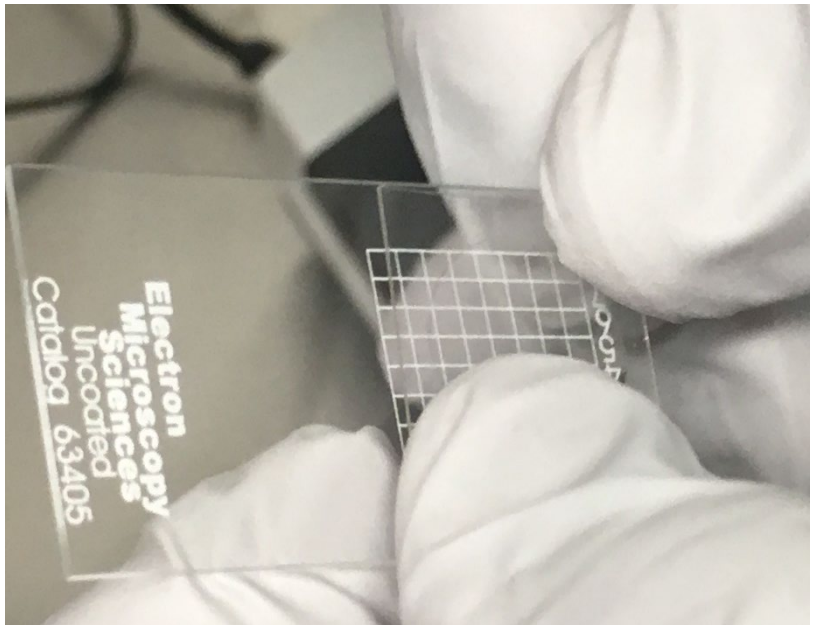


Simplified Test Protocol









Simplified Test Protocol

Test condition	Desired Result (# Beads collected)	Record of Your Results (# beads collected)
Clog/Deflection with containment devices “ON”	0	
Clog/Deflection with containment devices “OFF)	> 250	

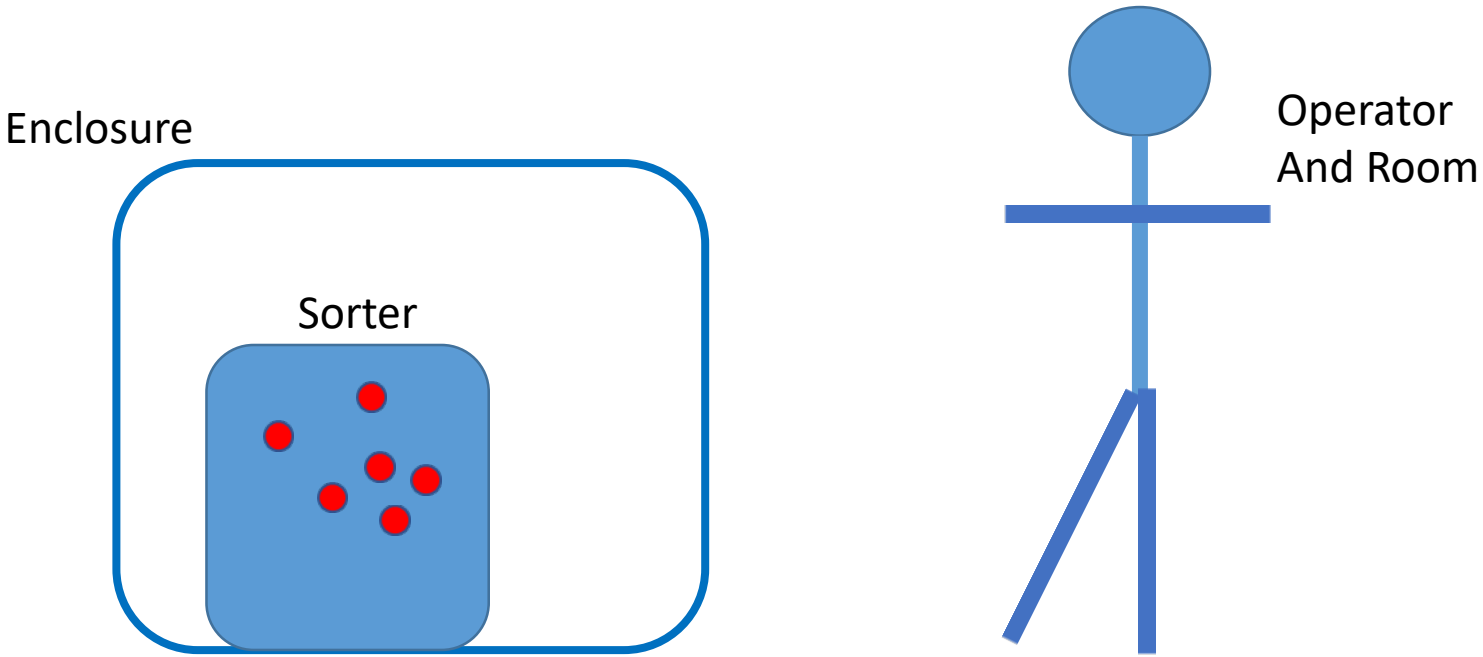
Other Recommended Tests

- Baseline for room
- If the sorter is within a containment enclosure
  - Take a sample outside of the enclosure while “ON”

- If you only have a portable aerosol management system (AMS) attached to your sorter and no enclosure
  - Test the exhaust air coming out of the AMS!
  - Portable (moved, bumped – good way to verify HEPA filter integrity in between annual tests)

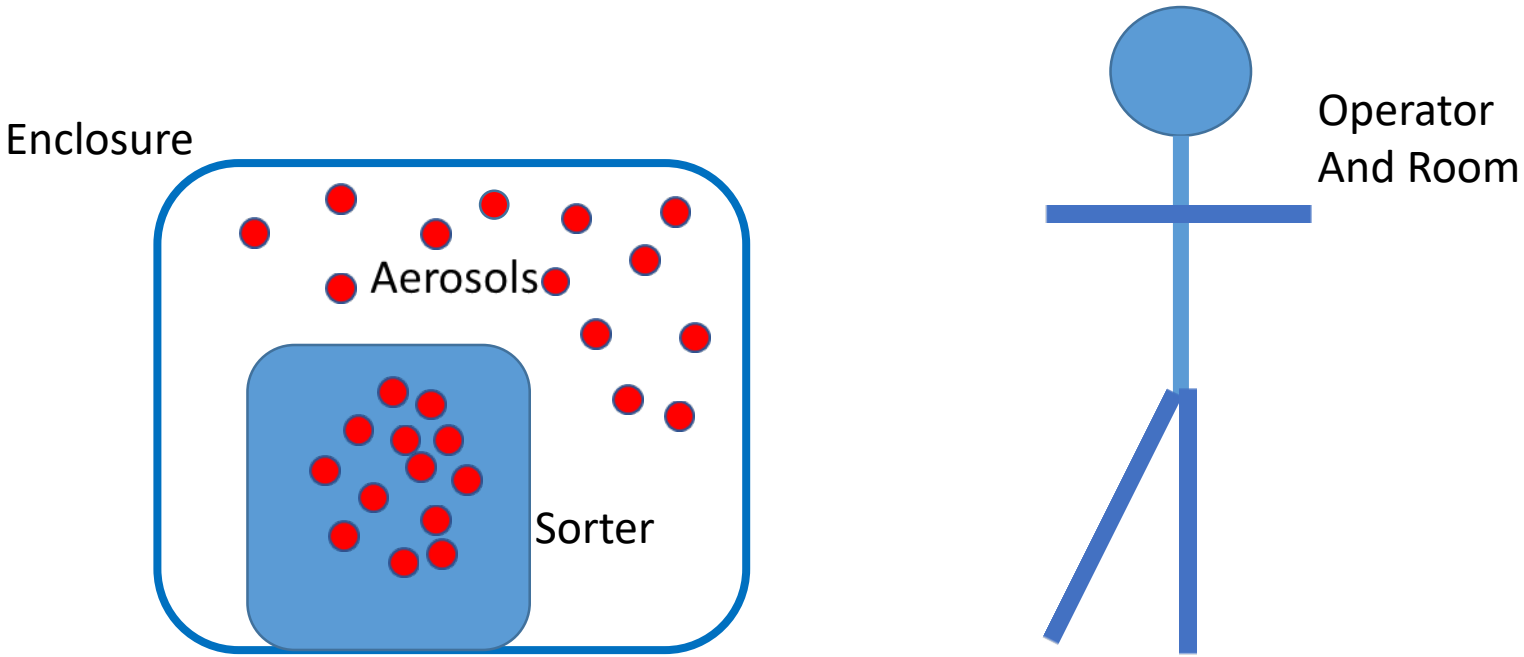
Aerosol Management System (AMS)

Testing to identify “where aerosols may go”



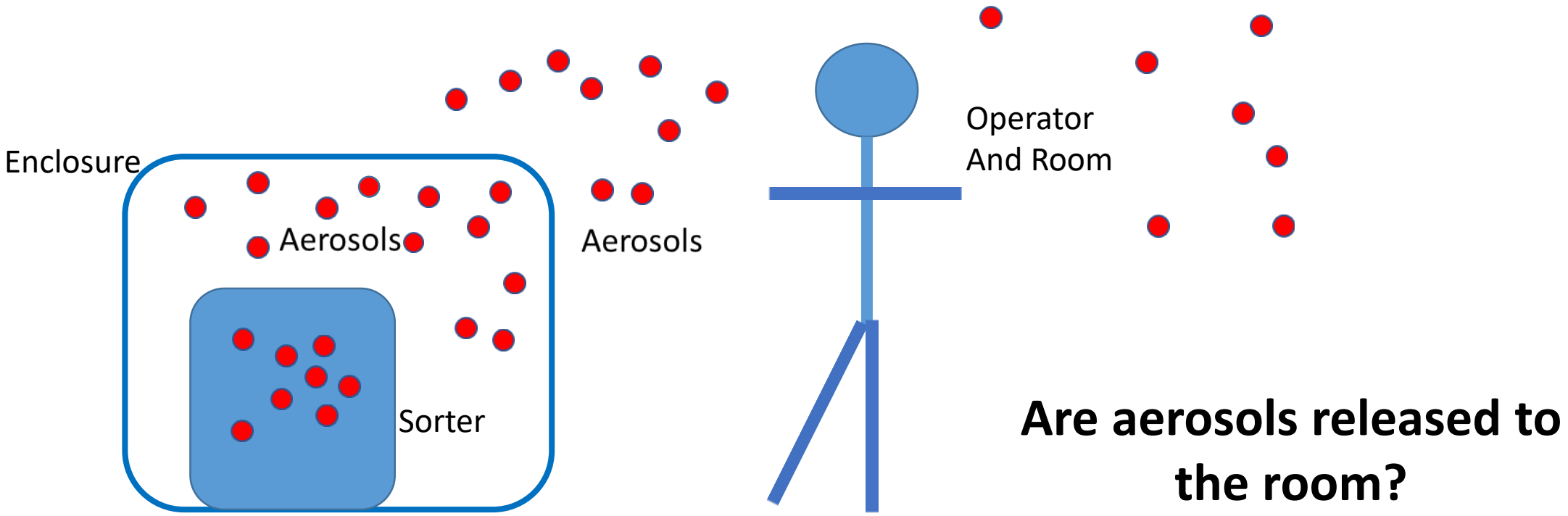
Are aerosols contained within the sorter?

Testing to identify “where aerosols may go”



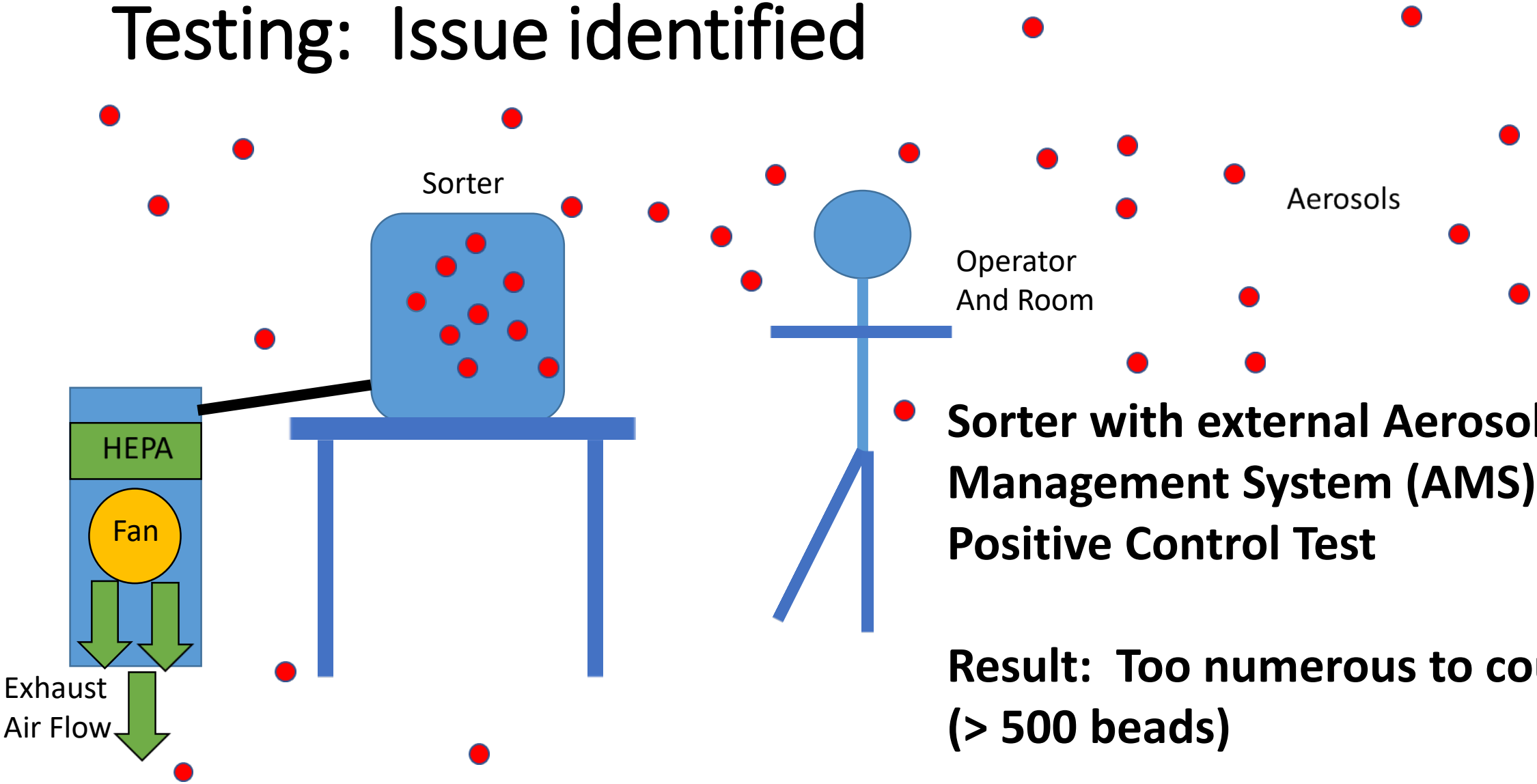
Are aerosols contained within the enclosure?

Testing to identify “where aerosols may go”



Are aerosols released to the room?

Testing: Issue identified

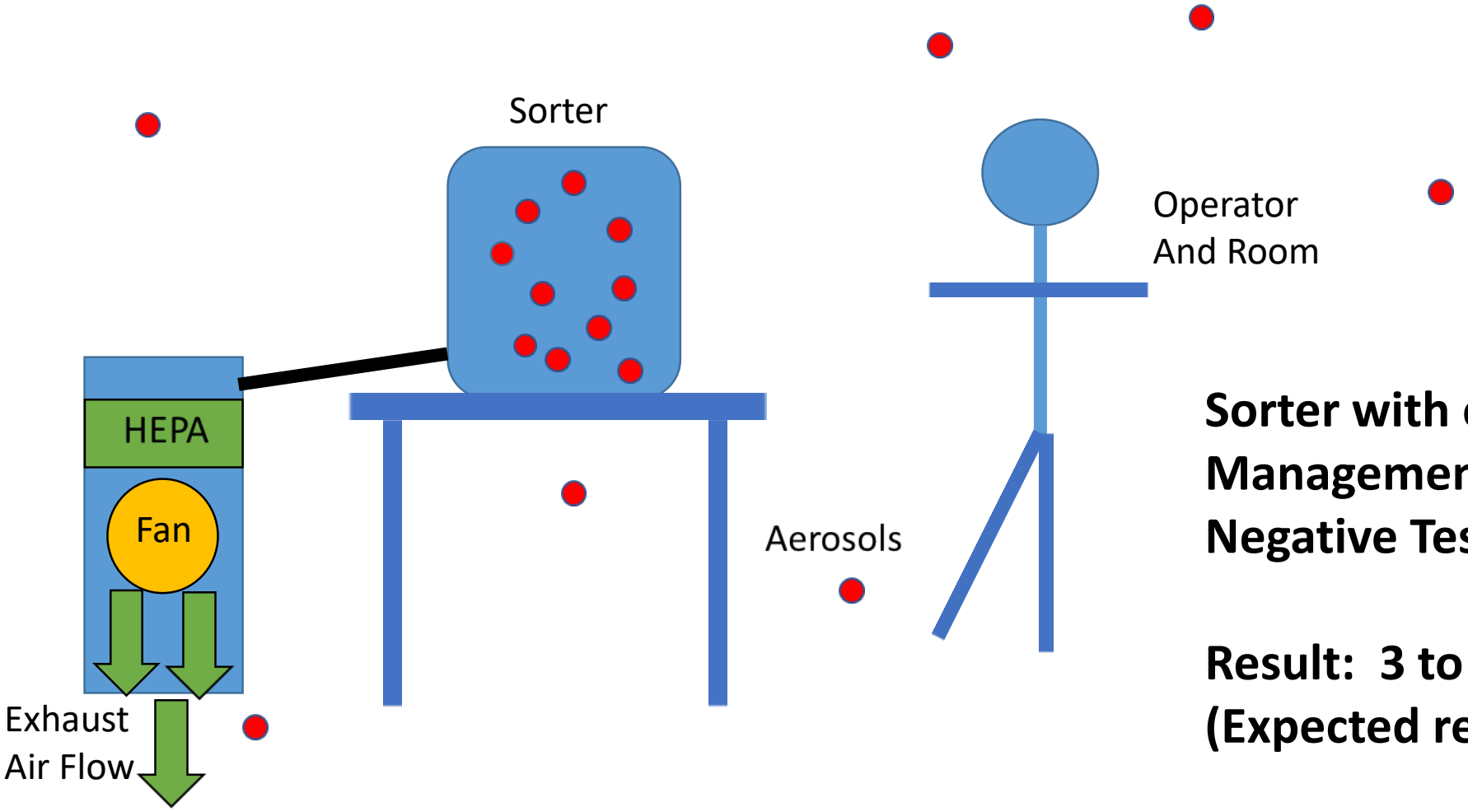


Sorter with external Aerosol Management System (AMS) OFF: Positive Control Test

Result: Too numerous to count (> 500 beads)

N-95 respirator needed!

Testing: Issue identified



Sorter with external Aerosol Management System (AMS) ON: Negative Test

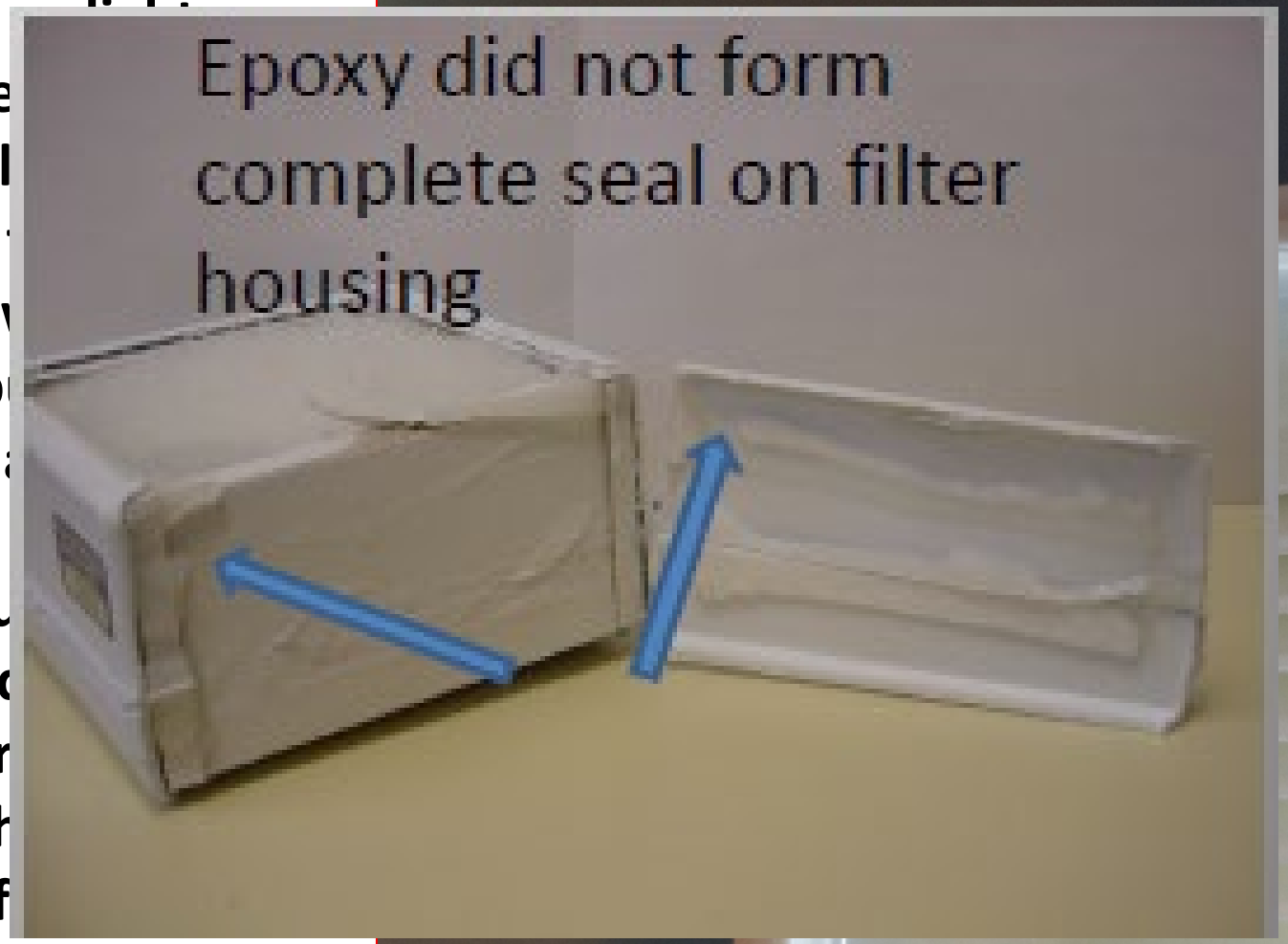
Result: 3 to 4 beads
(Expected result: ZERO beads)

Investigation: identified leak in HEPA filter

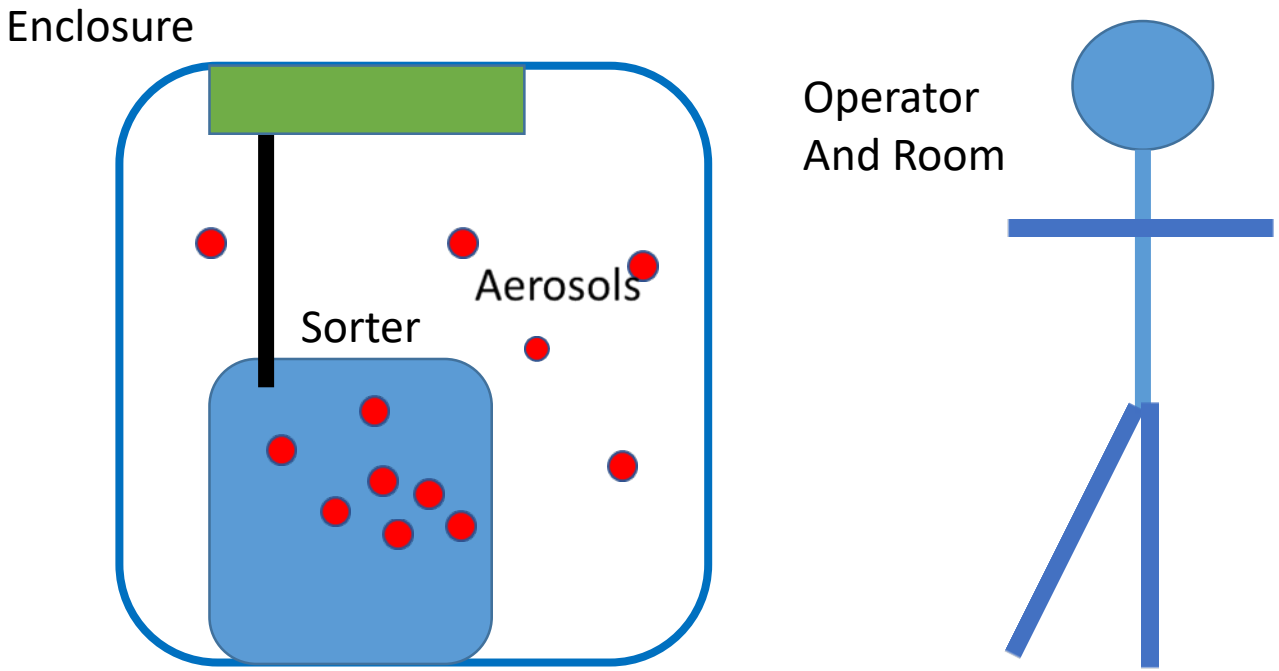
Test HEPA filters before use (after shipment, storage, moving)

Double Tested Filter

- Discovered
  dent on the
  the original
  packaging,
  not noted
  filter was p
  earlier for l
- The never u
  was opened
  slight separ
  expoxy/adh
  bonds the f
  plastic housing was
  discovered



Testing: Issue identified



Implications of this result:
Need to decontaminate the exterior of sorter and inside of the enclosure!

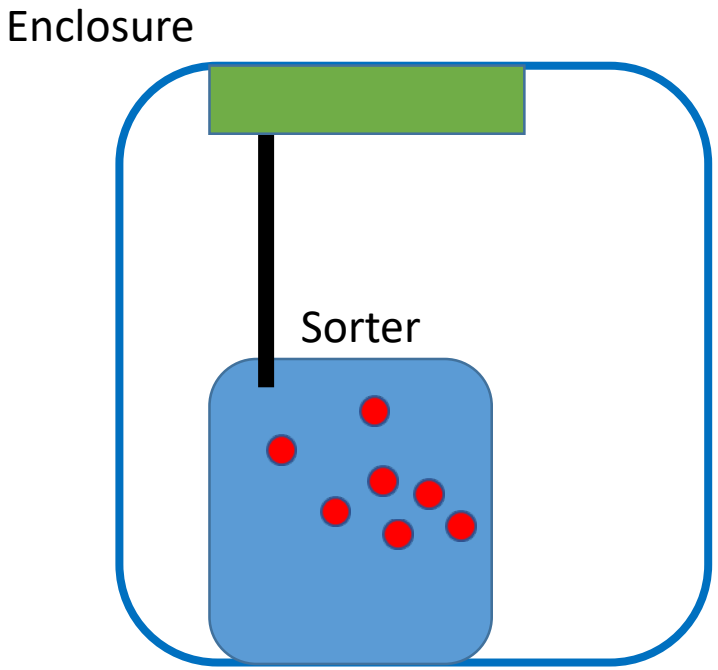
Sorter within Class I Biosafety Cabinet Enclosure:

Containment test with Enclosure "ON":

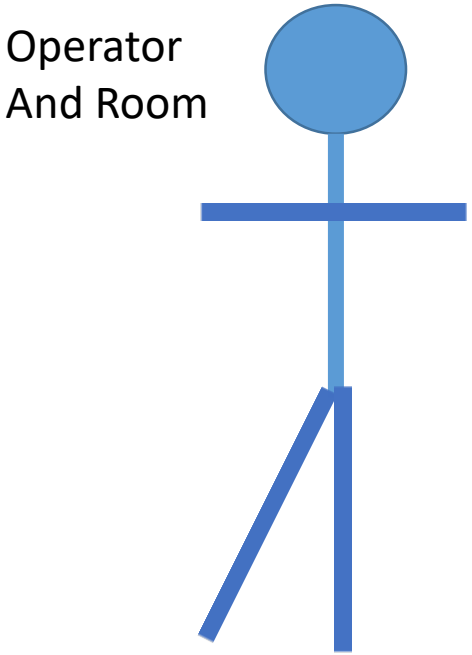
Bead result outside machine:
5 beads
Expected result: 0 beads

Notes: Beads either coming from attachment of exhaust tube to HEPA at top of enclosure
OR
From door gasket on front of sorter

Retesting after gasket correction



Implications for SOP:
Don't need to decontaminate sorter and inside enclosure



Implications for SOP:
Also tested "outside" sorter – 0 beads
Do not have to evacuate the room in the event of a clog or deflection

Sorter within Class I Biosafety Cabinet Enclosure:

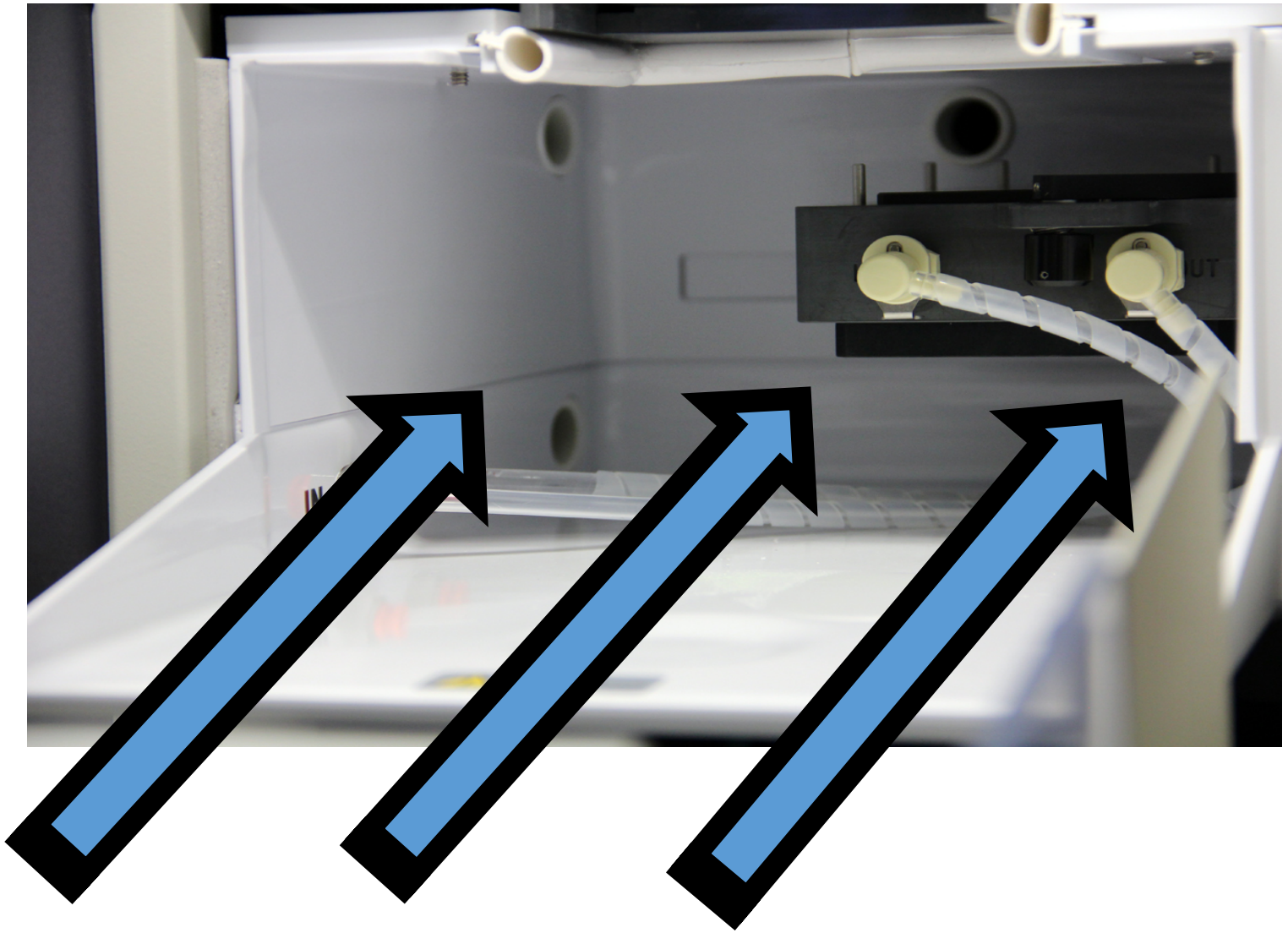
Containment test with Enclosure "ON":

Bead results:
Within Enclosure: 0 Beads

Outside Enclosure: 0 Beads

Retest results after addressing door gasket

High Air Changes per Hour (ACH) in Chamber (Airflow direction – away from operator)



Area = 0.3 ft3

Avg. Airflow velocity = 147 f/m
Area Opening = 0.34 ft2
CFM = 147 f/m x 0.34 ft2
CFM = 49.98

$$\text{ACH} = \frac{\text{CFM} \times 60}{\text{Area}}$$

$$\text{ACH} = \frac{49.98\ \text{CFM} \times 60\ \text{m/hr}}{0.3\ \text{ft3}}$$

ACH = 9,996

Note: Cell Sorter BSL3

Lab = 17 ACH

Filter Evaluation

Filter Evaluation

End of Presentation Slides
Questions?