A PERFORMANCE-BASED FAILURE MODE VALIDATION PROTOCOL FOR BSL-3 LABORATORIES

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Study Objectives

- Utilize an existing containment test from the flow cytometry community for BSL-3 lab validation
- Identify the extent of contamination drift from a "spill or release outside of primary containment ("SPORE-OOP-C") in normal & failure modes
- Verify the critical evacuation points (CEPs) for lab personnel in spill and worst-case scenarios (where is the safest location after a release?)

BSL-3 Facility Verification

- CDC/NIH BMBL 5th Edition
  - Section IV/BSL3/D.9.
    - "…The laboratory is designed such that under failure conditions the airflow will not be reversed."
  - Section V/ABSL3/D.6.
    - "…The ABSL-3 animal facility shall be designed such that under failure conditions the airflow will not be reversed."

BSL-3 Facility Verification

- DHHS CDC Select Agent Program Clarification Statements
  - "Documentation provided to demonstrate that under exhaust fan or power failure conditions, . . ., there is no reversal of air which originates within the BSL-3/ABSL-3 lab or vivarium room that travels all of the way outside the containment boundary."
  - "The BSL-3 anteroom is considered to be within the containment envelope."

DHHS CDC Select Agent Program Clarification Statements

- "A positive pressure excursion is not necessarily an airflow reversal;"

- "if a brief, weak positive pressure excursion is noted, a repeat test may be performed with airflow observation using an airflow indicator such as a smoke stick, or dry ice in a container of water, at the base of the closed laboratory door to confirm whether airflow reversal is occurring."

Pressure Readings Over Time During Failure Modes (Trending)

- No positive differential pressure readings
  - Congrats!
- Does it reflect reality?
- What are we evaluating?
  - Worst-case event
  - Spill or release at time of HVAC Failure
    - (Why redundancy is critical)
- Researchers evacuating laboratory
  - Opening exit doors in immediate aftermath of release

What Would You Want to Know about your BSL-3 Lab?

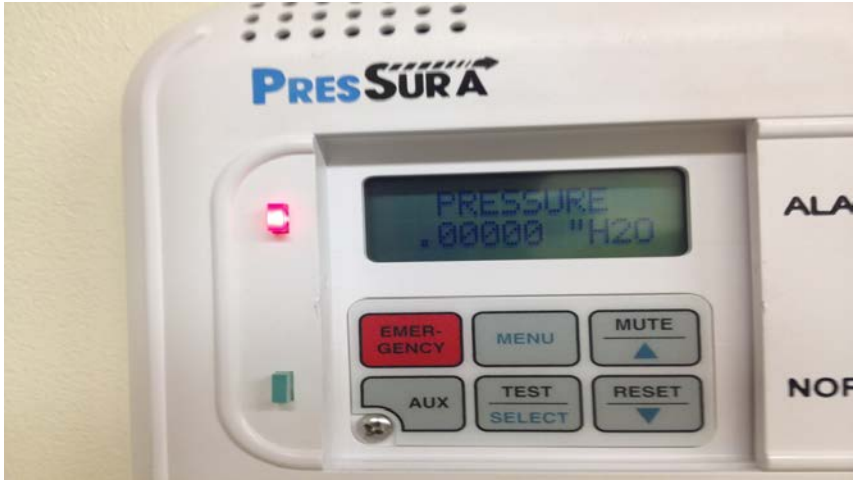
- Does the facility keep aerosols created during a spill within the BSL-3 lab during failure?
- Will exiting the laboratory immediately after a spill carry aerosols out to the anteroom?
- Under static conditions, what is the impact of opening/closing doors adjacent to the BSL-3 lab?
- Where is the CRITICAL EVACUATION POINT (CEP)?
  - Location where aerosols don't spread to.

Failure Mode Testing

Neutrality Observed During Failure Testing

Magnehelic Gauges Read Neutral

Weak Positive Pressure Excursions



- True Neutral?
  - (0.00000)
- + 1/100,000th inch H2O
  - (+0.00001"H2O)

Differential Pressure Readings (A Gold Standard?)

Monitoring pressure at the door during failure test

Manpower or machine at each entry

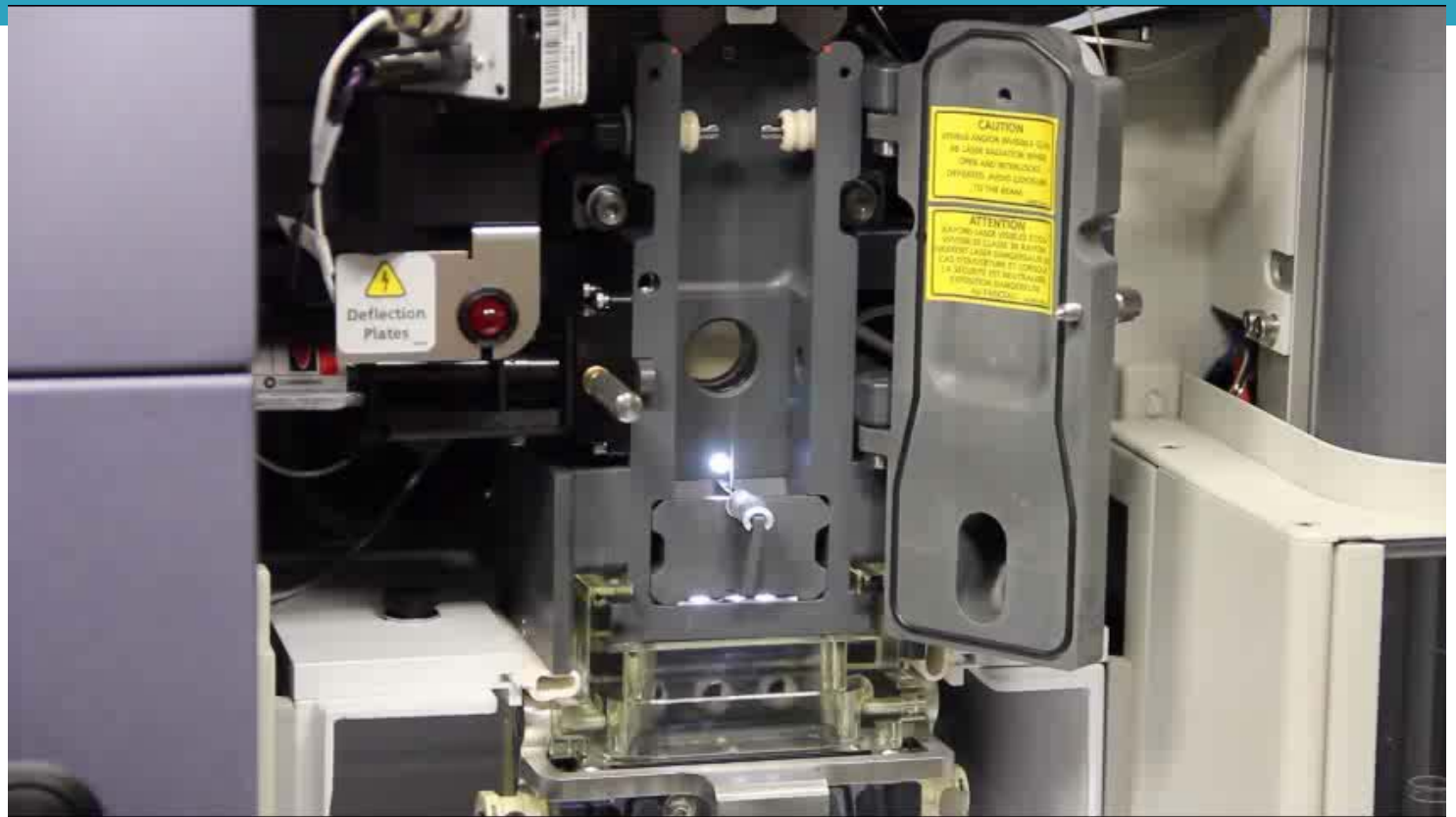
Road to our test

- Heavy smoke test to identify leak points for sampler placement
- Light smoke release challenge as a validation test (our likely spills will not be continuous releases)
- Review of our spill history (n=2 in 18 yrs)
- Modification of cell sorting containment test

An emergency condition . . .

Made more significant . . .

Site-Specific Assessment of Worst-Case Failure Scenario



Biohazard Release References

- Kenny and Sabel
  - Dropped 500 ml flask (1.4 x 10^12 *Serratia marcescens* cells) from 20 inches in chamber
    - **54,285 viable S. marcescens/m3**
      - Kenny, M.T., and Sabel, F.L. (1968) "Particle Size Distribution of *Serratia marcescens* Aerosols Created During Common Laboratory Procedures and Simulated Laboratory Accidents."
        - Sampling air from tightly sealed chamber
    - Identified small particle aerosols (most in range of 1 to 7.5 um size)

Biohazard Release References

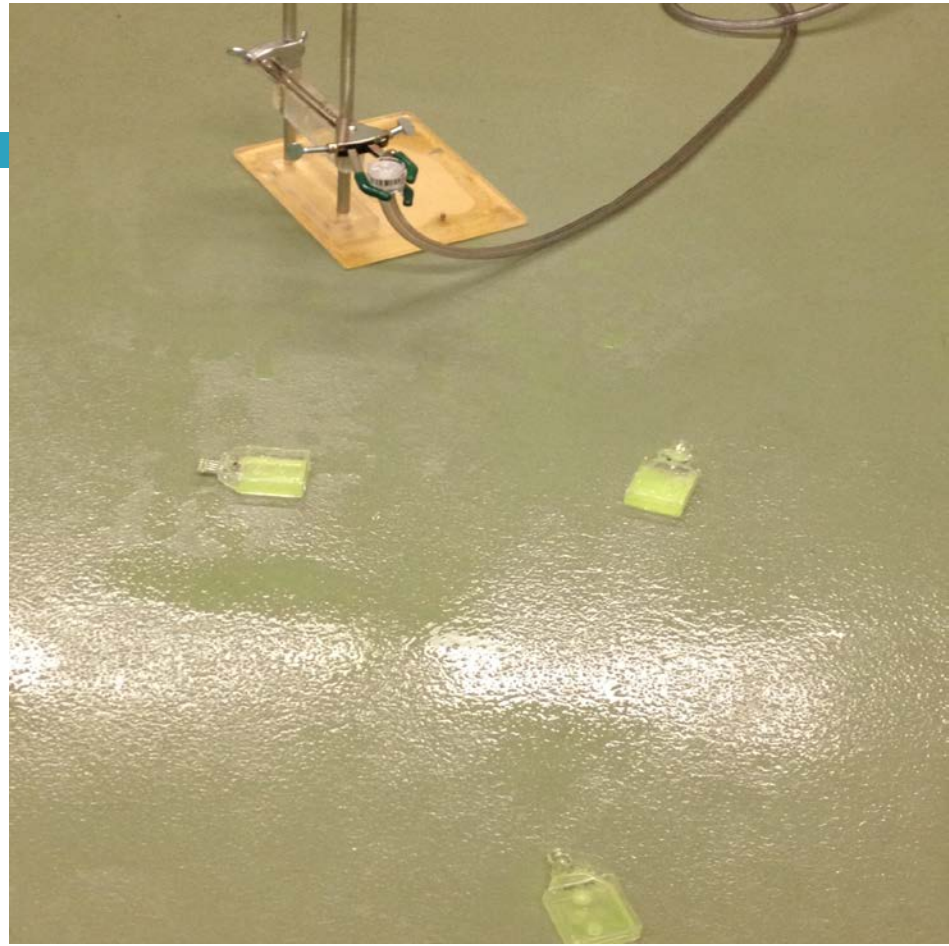
- Bennett and Parks (2005)
  - Use of Potassium Iodide aerosol tracer test used for testing biosafety cabinets to quantify BSL-3 lab protection capabilities.
    - Importance of anterooms verified
    - Volume of inflow air more important that pressure
    - Opening/closing doors will disseminate particles from the spill area to the anteroom and beyond
      - Bennett, A.M., Parks, S.R., and BenBoug, J.E. (2005) Development of Particle Tracer Techniques to Measure the Effectiveness of High Containment Laboratories. Applied Biosafety, 10(3) pp 139-150.

Biohazard Release References

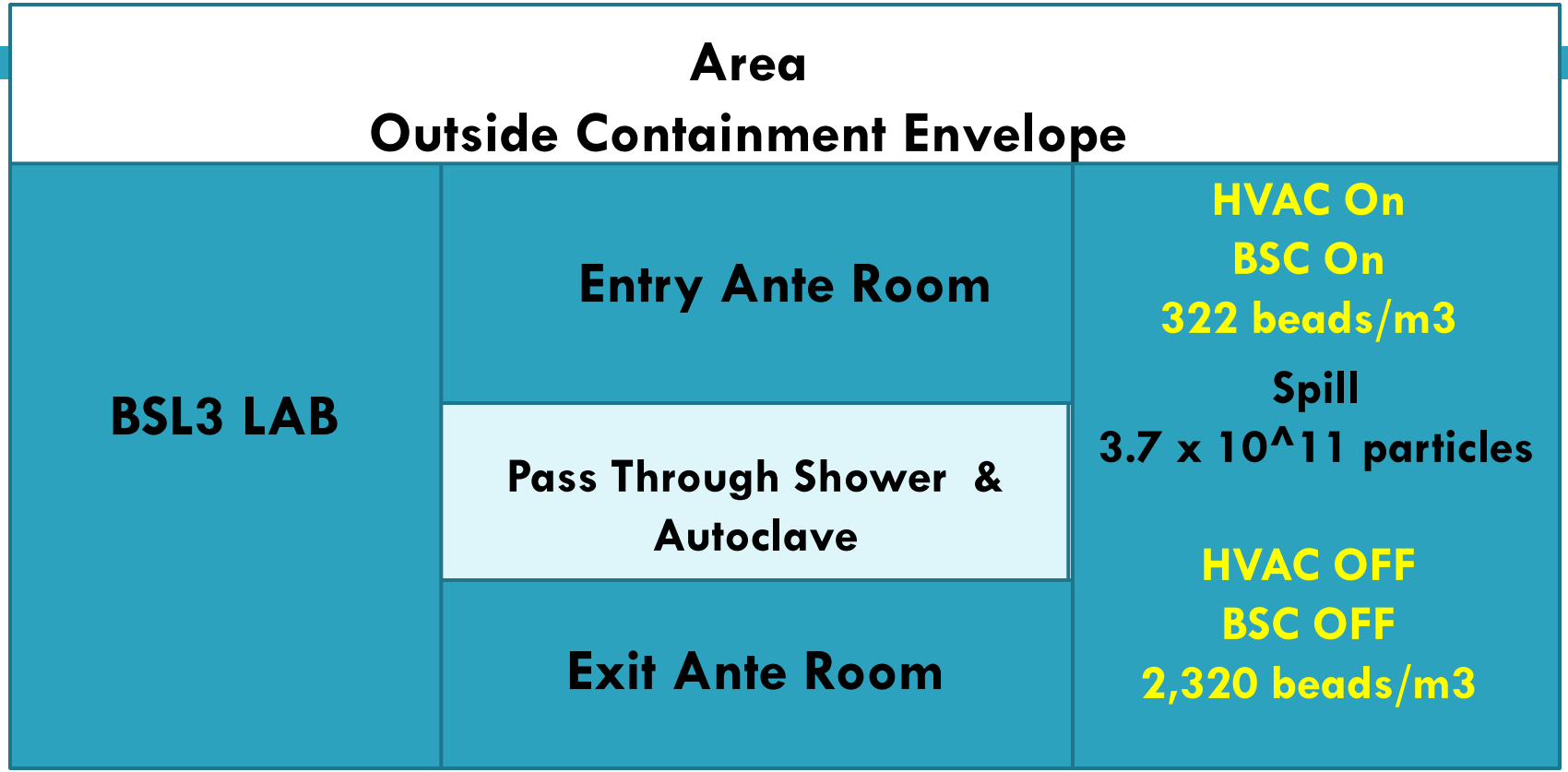
- Bennett and Parks (2006)
  - 13 Different release scenarios in small BSL-3 lab, with anteroom, and general access corridor
    - All experiments with ventilation system OFF
    - Recovered high # viable organisms in small particle size range
      - 1,000 – 10,000's of CFU/m3 recovered (*Bacillus atrophaeus*)
        - Bennett, A. and Parks, S. (2006), Microbial Aerosol Generation During Laboratory Accidents and Subsequent Assessment. Journal of Applied Microbiology, 100: 658-663.

Fluorescent Beads

- Small uniform particles
- 0.5, 2.0 um
- 10^11 particles/ml
- Use in FACS failure tests
- Can gauge spread of contamination
- Can obtain results instantly
- Easy to clean
- Inexpensive
- T25 Tissue Culture flasks, 50 ml
  - Spill mixture: ONE 50 ml flask filled with 1 ml 0.5 um beads + 14 ml PBS, and TWO 50 ml flasks each with 1 ml 2.0 um beads + 14 ml PBS.



Fluorescent Bead Release Test
Normal & Failure Conditions

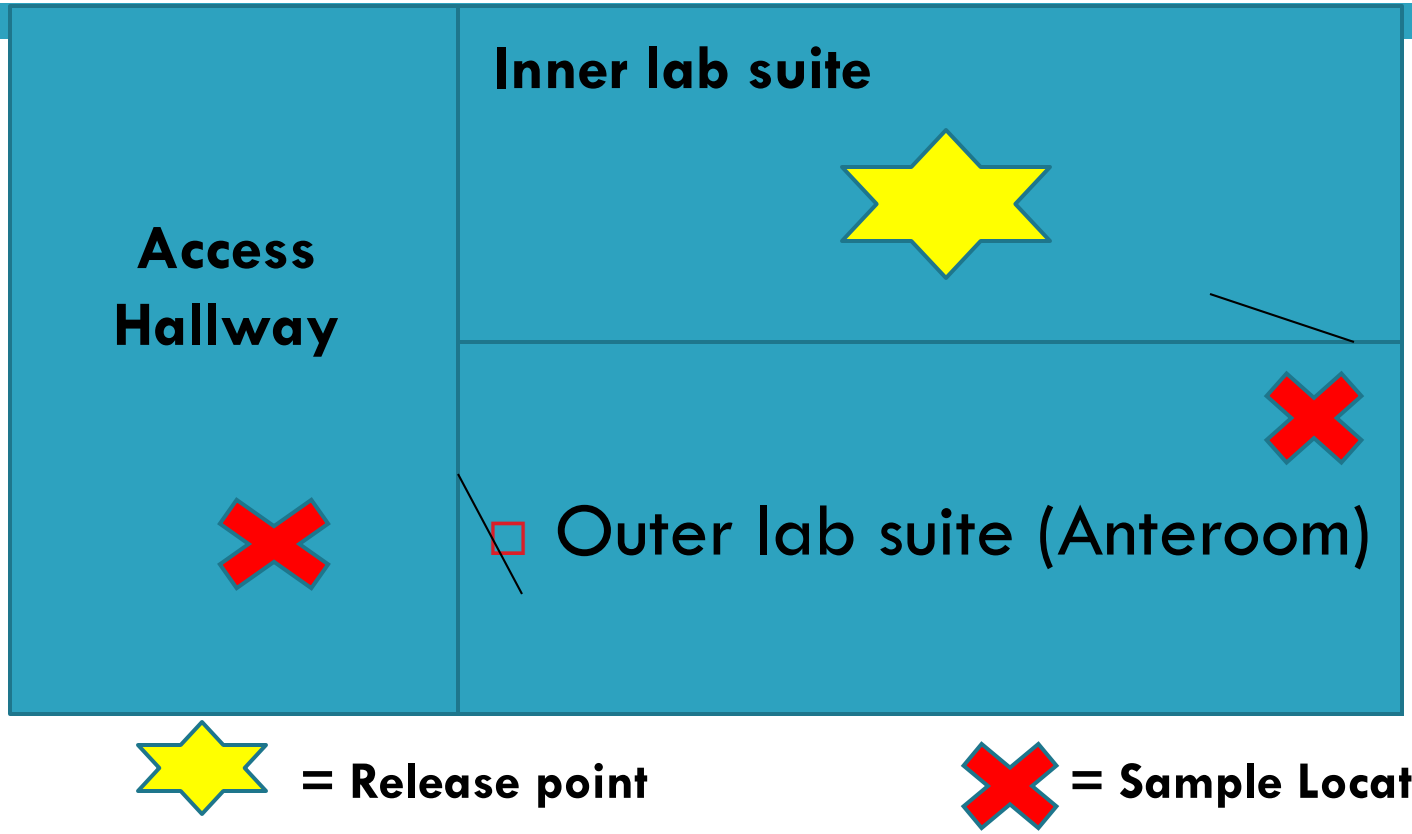


Test Lab Descriptions

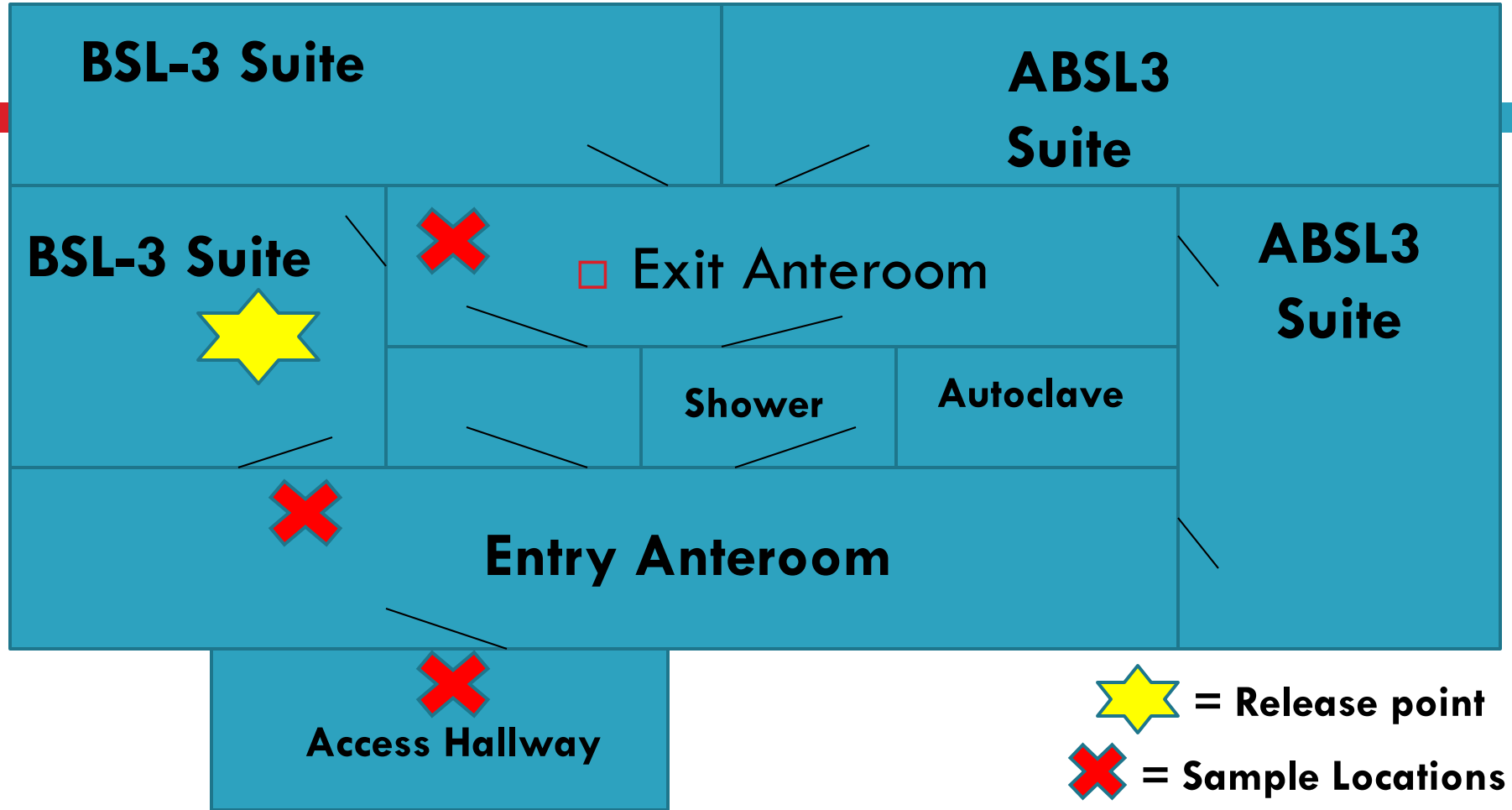
TEST LOCATION	LAB DESCRIPTION
#1 – HVAC on BSL2-Enhanced Lab	Non-airborne pathogen use, outer lab as anteroom (Spill with lab exit w/ normal HVAC, BSC on)
#2 - HVAC on New BSL3	Airborne pathogen use, modern enhanced BSL3 (Spill with lab exit w/normal HVAC, BSC on)
#3 – HVAC off Old ABSL3	Not in use, Exhaust/Supply interlock pneumovalve system (Spill with lab exit w/ exhaust failure, BSC off)
#4 – HVAC off New BSL3	Airborne pathogen use, modern enhanced BSL3 (Spill with lab exit w/ exhaust failure, BSC off)
#5 – HVAC off Old BSL3	Non-airborne pathogen use, exhaust/supply interlock damper, with supply air diverted (Spill with lab exit w/Exhaust failure, BSC off)

cyclex-d cassette and differential pressure meter

Location #1: BSL2-Enhanced Suite

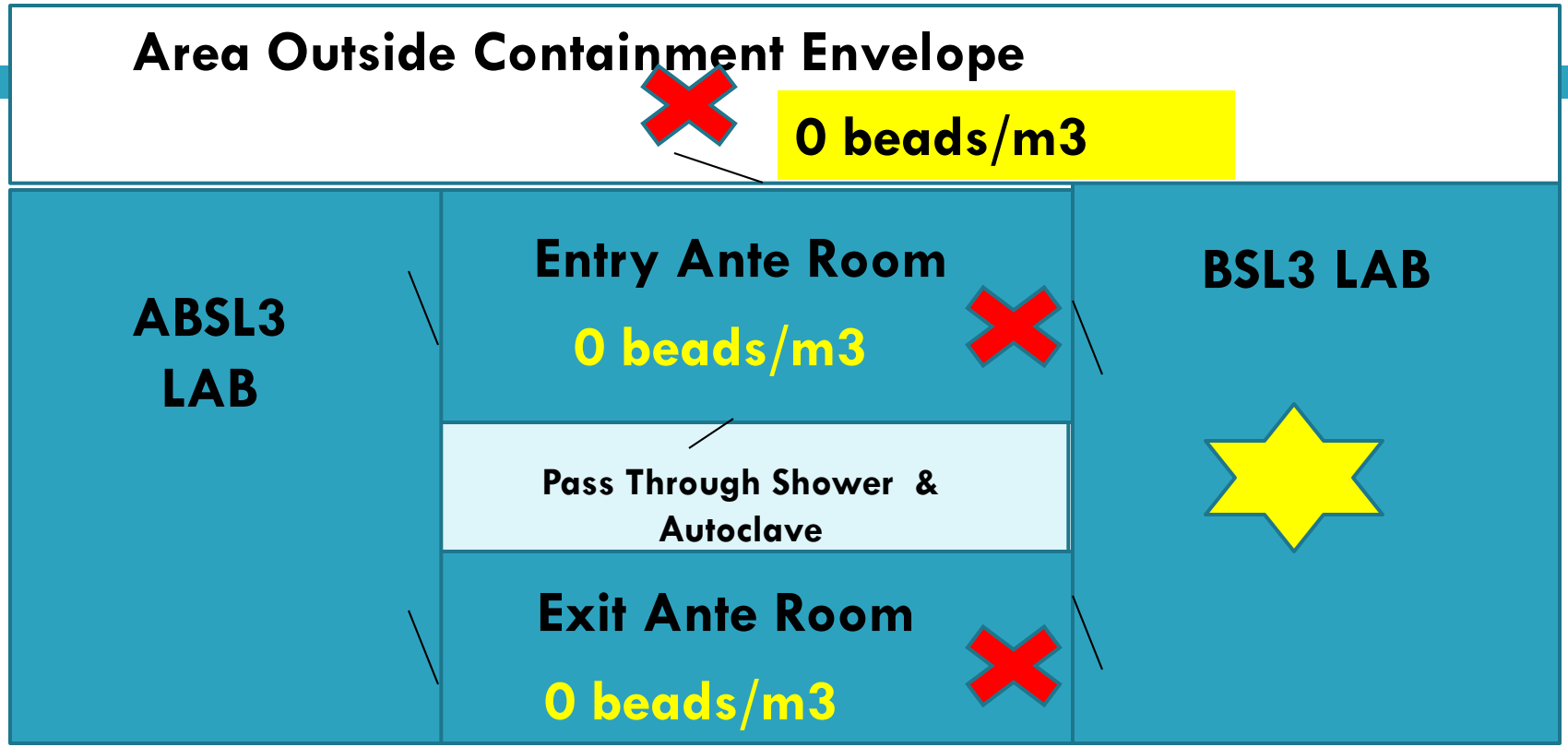


Location #2: Modern BSL3 Lab (HVAC On)

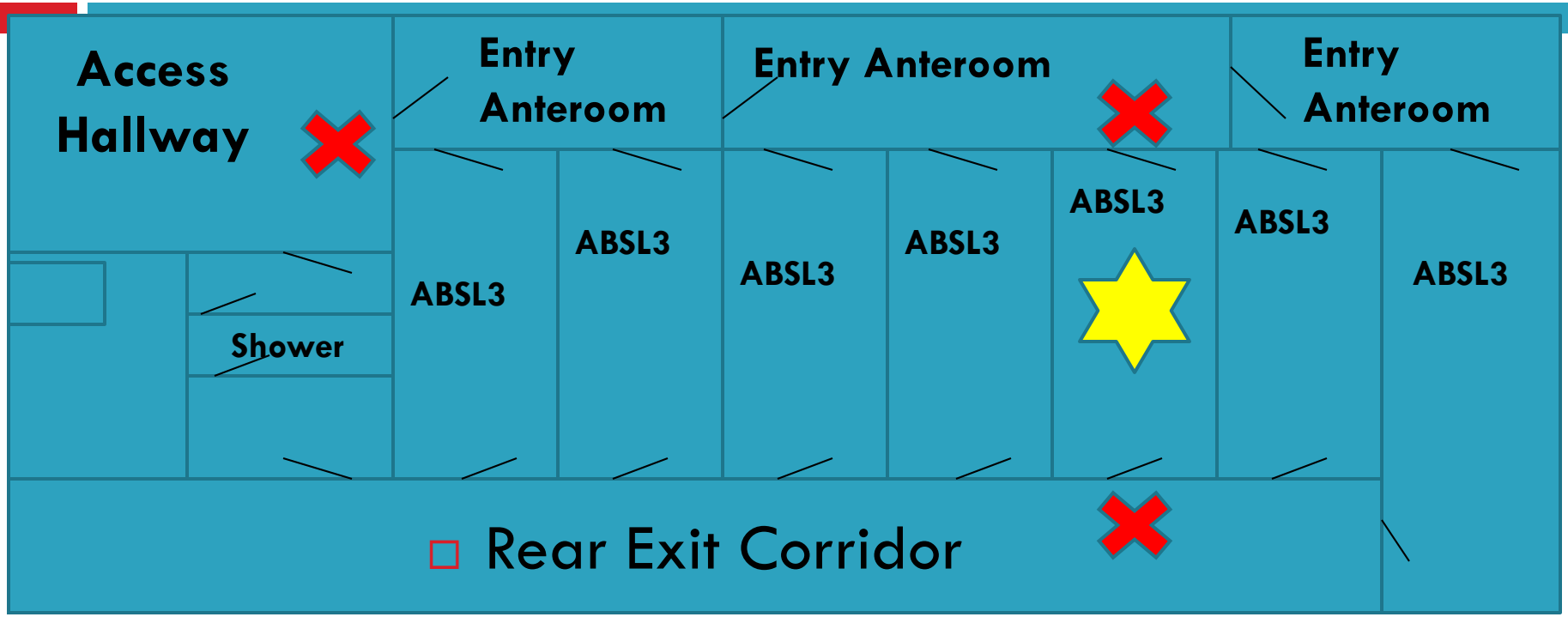


Location #4: Fluorescent Bead Release Test

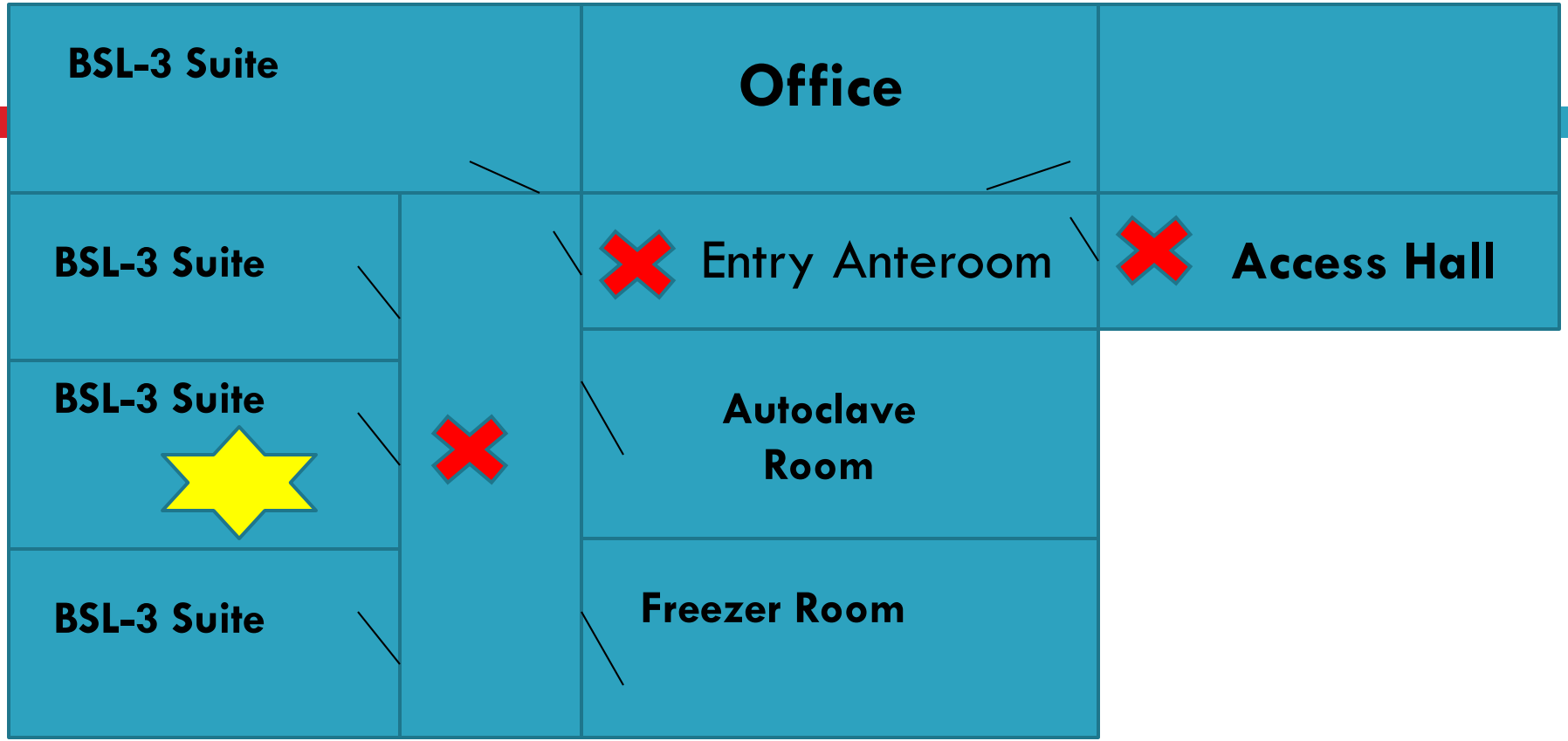
Fan Failure Test/BSC OFF w/Lab Exit



Location #3: Old ABSL3 Lab



Location #5: Old BSL3 Lab



= Release point

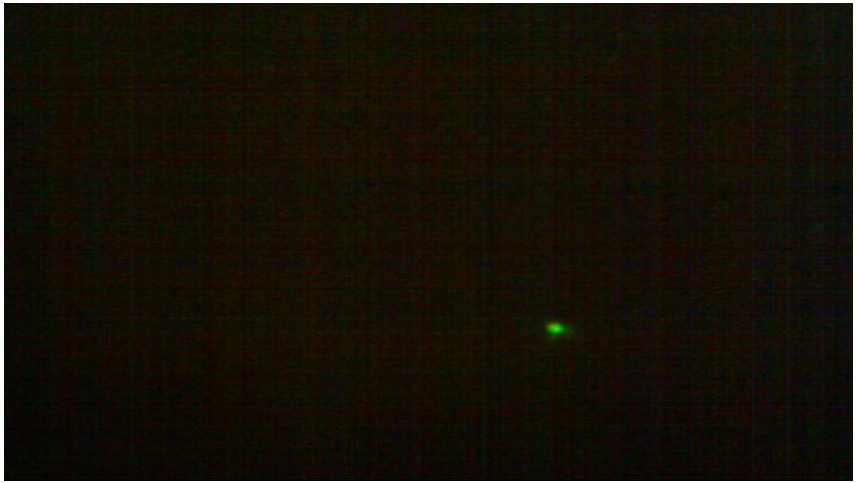
= Sample Locations

RELEASE TEST SAMPLING DATA
(Total Beads: 1 bead = 10 particles/m3)

Sample Location	Sample Time Point	Location 1 BSL2 + (Normal)	Location 2 New BSL3 (Normal)	Location 3 Aged ABSL3 (Failure)	Location 4 New BSL3 (Failure)	Location 5 Aged BSL3 (Failure)
Exit Anteroom	Baseline	0	1	0	0	0
	0' – 5'	0	0	0	0	0
	10' -15'	0	0	0	0	0
	20'- 25'	0	0	0	0	0
	30'-35'	1	0	0	0	0
Entry Anteroom	Baseline		0	0	0	1
	0' – 5'		0	0	0	1
	10' -15'		0	0	0	0
	20'- 25'	N/A	0	0	0	0
	30'-35'		0	0	0	0
Access Hallway	Baseline	0	0	0	0	0
	0' – 5'	0	0	0	0	1
	10' -15'	0	0	0	0	0
	20'- 25'	0	0	0	0	0
	30'-35'	0	0	0	0	0

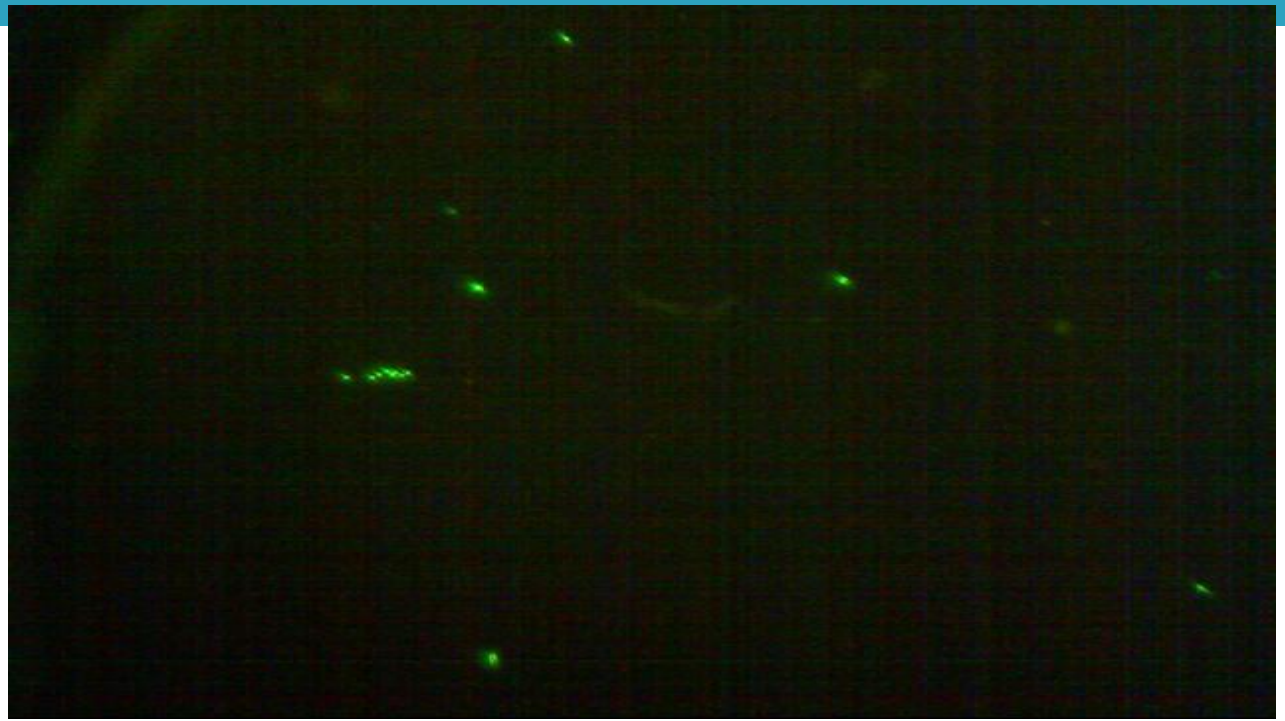
Sample Images

- **Positive Single Bead**



- **Negative Filter Result**

Image from Release Point



Results summary

- Zero contamination identified outside of containment in modern BSL3 labs
- No beads identified outside of old ABSL3 room (verification of Bennett/Parks anteroom study)
- Single beads identified in old BSL3 and BSL2-enhanced labs were likely contaminants
- Aged BSL3 facilities offered similar containment

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"It Takes A Village"

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  - Tim Acker
  - Darryl Redding
  - Mattison Finkle
  - George Hotchkiss
  - Chris Scranton

Equipment and Supplies

- Beads from POLYSCIENCES, INC.:
  - Catalogue # 17152-10: Fluoresbrite Yellow Green (YG) 0.5 um latex Microspheres, 3.64×10^{11} particles/ml, 10 ml/vial packaged as 2.5% aqueous suspension
  - Catalogue # 18338-5: Fluoresbrite Yellow Green (YG) 2.0 um latex Microspheres, 5.68×10^{9} particles/ml, 5 ml/vial packaged as 2.5% aqueous suspension
- Phosphate buffered saline
- T25 Tissue Culture flasks, 50 ml
  - Spill mixture: 1 50 ml flask filled with 1 ml 0.5 um beads + 14 ml PBS, and 2 50 ml flasks each with 1 ml 2.0 um beads + 14 ml PBS.
- cyclex-d filter cassettes (disposable bioaerosol impaction sampler), SKU: 120135, environmental monitoring systems

Equipment and Supplies

- Air Pump: GAST Model 10-709 (Operated at 20 LPM for cyclex-d cassettes, 28.3 LPM for Anderson Impaction Sampler)
  - Gilibrator-2 Air Flow Calibrator, Sensidyne Industrial Health & Safety Equipment
- Shortridge Multimeter ADM-880C, Shortridge Instruments, Inc.
- Smoke Test
  - Roscoe Fog Machine, Model #OMEGA XT
  - TSI, Inc. DustTrak II, Model 8532
  - TSI, Inc. AeroTrak Handheld Particle Counter, Model 9303

Thank You!

- Questions after presentations