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

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ABSA Conference
Kansas City, Missouri
October 23, 2013
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.”
  
  - “...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.”
“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.”
<table>
<thead>
<tr>
<th>Question</th>
<th>Answer</th>
</tr>
</thead>
<tbody>
<tr>
<td>No positive differential pressure readings</td>
<td><strong>Congrats!</strong></td>
</tr>
<tr>
<td>Does it reflect reality?</td>
<td><strong>Worst-case event</strong></td>
</tr>
<tr>
<td>What are we evaluating?</td>
<td><strong>Spill or release at time of HVAC Failure</strong></td>
</tr>
<tr>
<td></td>
<td><em>Why redundancy is critical</em></td>
</tr>
<tr>
<td>Researchers evacuating laboratory</td>
<td><strong>Opening exit doors in immediate aftermath of release</strong></td>
</tr>
</tbody>
</table>
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
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)
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 than pressure
  - Opening/closing doors will disseminate particles from the spill area to the anteroom and beyond

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*)

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

<table>
<thead>
<tr>
<th>Area</th>
<th>Outside Containment Envelope</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BSL3 LAB</strong></td>
<td>HVAC On</td>
</tr>
<tr>
<td></td>
<td>HVAC OFF</td>
</tr>
<tr>
<td>Entry Ante Room</td>
<td>BSC On</td>
</tr>
<tr>
<td></td>
<td>BSC OFF</td>
</tr>
<tr>
<td>Pass Through Shower &amp; Autoclave</td>
<td>322 beads/m³</td>
</tr>
<tr>
<td></td>
<td>3.7 x 10^{11} particles</td>
</tr>
<tr>
<td>Exit Ante Room</td>
<td>Spill</td>
</tr>
<tr>
<td></td>
<td>2,320 beads/m³</td>
</tr>
</tbody>
</table>
## Test Lab Descriptions

<table>
<thead>
<tr>
<th>TEST LOCATION</th>
<th>LAB DESCRIPTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>#1 – HVAC on BSL2-Enhanced Lab</td>
<td>Non-airborne pathogen use, outer lab as anteroom (Spill with lab exit w/ normal HVAC, BSC on)</td>
</tr>
<tr>
<td>#2 - HVAC on New BSL3</td>
<td>Airborne pathogen use, modern enhanced BSL3 (Spill with lab exit w/normal HVAC, BSC on)</td>
</tr>
<tr>
<td>#3 – HVAC off Old ABSL3</td>
<td>Not in use, Exhaust/Supply interlock pneumovalve system (Spill with lab exit w/ exhaust failure, BSC off)</td>
</tr>
<tr>
<td>#4 – HVAC off New BSL3</td>
<td>Airborne pathogen use, modern enhanced BSL3 (Spill with lab exit w/ exhaust failure, BSC off)</td>
</tr>
<tr>
<td>#5 – HVAC off Old BSL3</td>
<td>Non-airborne pathogen use, exhaust/supply interlock damper, with supply air diverted (Spill with lab exit w/Exhaust failure, BSC off)</td>
</tr>
</tbody>
</table>
cyclex-d cassette and differential pressure meter
# Location #1: BSL2-Enhanced Suite

<table>
<thead>
<tr>
<th>Access Hallway</th>
<th>Inner lab suite</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>□ Outer lab suite (Anteroom)</td>
</tr>
</tbody>
</table>

- = Release point
- = Sample Locations
Location #4: Fluorescent Bead Release Test
Fan Failure Test/BSC OFF w/Lab Exit

Area Outside Containment Envelope

- 0 beads/m³

<table>
<thead>
<tr>
<th>ABSL3 LAB</th>
<th>Entry Ante Room</th>
<th>Exit Ante Room</th>
<th>BSL3 LAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 beads/m³</td>
<td>0 beads/m³</td>
<td>0 beads/m³</td>
<td></td>
</tr>
</tbody>
</table>

Pass Through Shower & Autoclave

= Release point
= Sample Locations
Location #3: Old ABSL3 Lab

Access Hallway

Entry Anteroom

Entry Anteroom

Entry Anteroom

Rear Exit Corridor

= Release point

= Sample Locations
Location #5: Old BSL3 Lab

- BSL-3 Suite
- Entry Anteroom
- Access Hall
- Autoclave Room
- Freezer Room

= Release point
= Sample Locations
## RELEASE TEST SAMPLING DATA
(Total Beads: 1 bead = 10 particles/m³)

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

- Positive Single Bead
- Negative Filter Result
Image from Release Point
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
Acknowledgements

“It Takes A Village”

- Yale EHS
  - Brian Mullins
  - Maryjo Lanzillotta
  - James D’Addio
  - Miguel Berrios
  - Doug Noble
  - Partha Krishnan
  - Shumin Bian
  - Daniel Ormrod

- BSL-3 Facility
  - Rachel Ardito
  - Laurie Lewkowicz
  - Rob Durant

Thank You!

- Flow Cytometry Containment Core
  - Geoffrey Lyon

- Yale Facilities
  - Larry Busillo
  - Jack Tiboni
  - Arthur Pocevic
  - 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 x 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 x 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