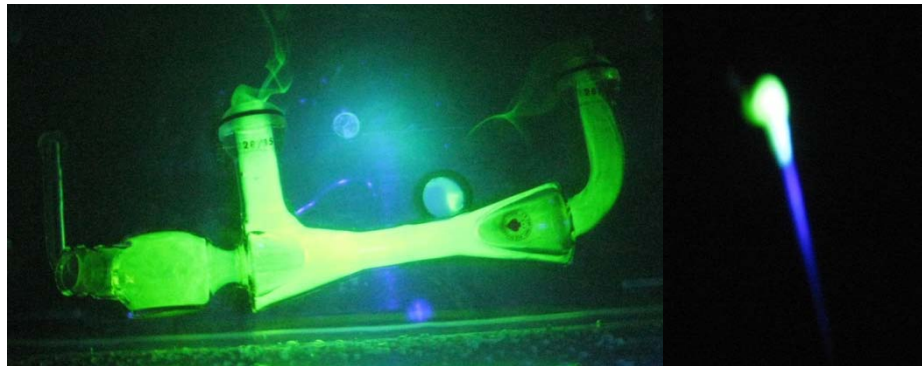


FLUORESCCEIN ANALYSIS OF AN AEROSOL EXPOSURE CHAMBER



American Biological Safety Association Conference 2012

October 24th

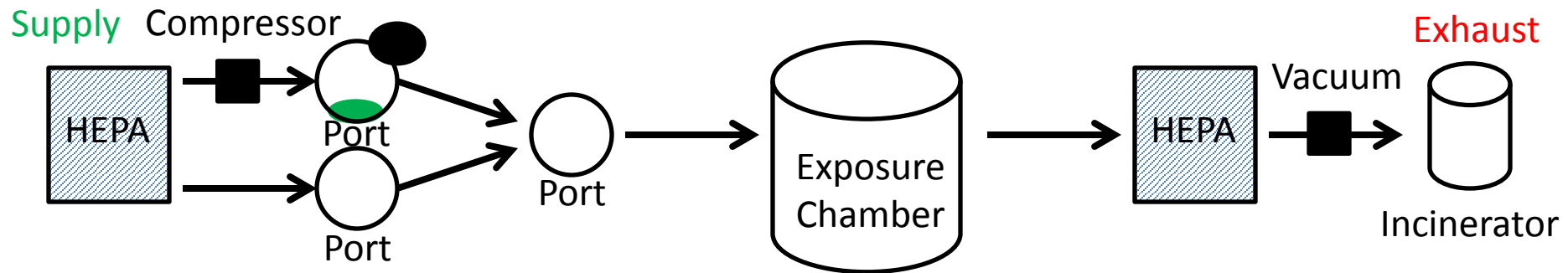
Thomas Cremer, Ph.D.

Presentation Disclaimer

- This applied biosafety study reviews a process for testing aerosol generating equipment.
- The goal is to provide data-driven risk assessments.
- This study does not promote or discourage the use of any particular model or manufacture of aerosol exposure chambers.
- The views in this presentation do not necessarily represent those of my past or current employer.
- Each institution or organization must conduct their own risk assessment regarding the hazards and mitigating measures for any procedure. Consult with the BMBL 5th edition.

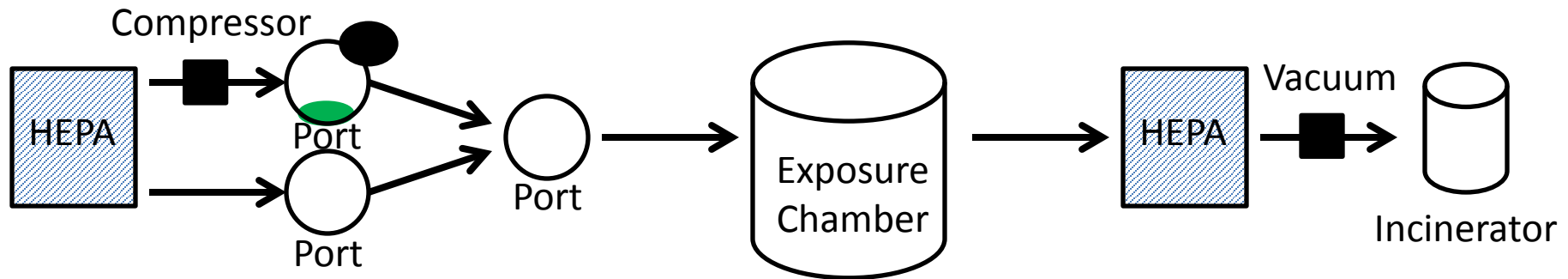
Aerosol Exposure System

- Contained system to produce aerosols for deep-lung infections
 - Includes RG 3 organisms: *Yersinia*, *Francisella*, *Mycobacteria*
- System capacity: 100 mice, 15-20 guinea pigs, 5 rabbits



Aerosol Exposure System

- No annual certification as a primary containment barrier
- Too large to fit within a biosafety cabinet



Safety Features & Concerns

Safety features

- HEPA filtered supply & exhaust
- Chamber under negative pressure
- Post-HEPA incinerator (1450° F)
- UV decontamination lights
- Must be sealed to start

Safety concerns

- Glass nebulizer is used
- Aerosolization outside BSC
- No annual certification
- UV decontamination is limited
- System integrity over time?
- Decontamination methods?
- SOP may include sharps

Reasons For The Study

- Aerosol system used in ABSL-3
 - Initial protocol review
- Aerosolized agents
 - *Mycobacterium tuberculosis*
 - No select agents
- Previous NIOSH study
 - Air sampling for *M. tb.*
 - Results negative
 - Limit of detection/sensitivity?

This Health Hazard Evaluation (HHE) report and any recommendations made herein are for the specific facility evaluated and may not be universally applicable. Any recommendations made are not to be considered as final statements of NIOSH policy or of any agency or individual involved. Additional HHE reports are available at: <http://www.cdc.gov/niosh/hhe/reports>



NIOSH HEALTH HAZARD EVALUATION REPORT

HETA #2004-0081-3002
New York University School of Medicine
New York City, New York

June 2006

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Centers for Disease Control and Prevention
National Institute for Occupational Safety and Health



Experimental Design & Sampling Methods

- Simple and adaptable
- Materials required:
 - Fluorescein
 - Woods lamp/black light
 - Water
 - Paper towels

Advanced materials:

- Fluorometer (quantitative results)
- Collection swabs



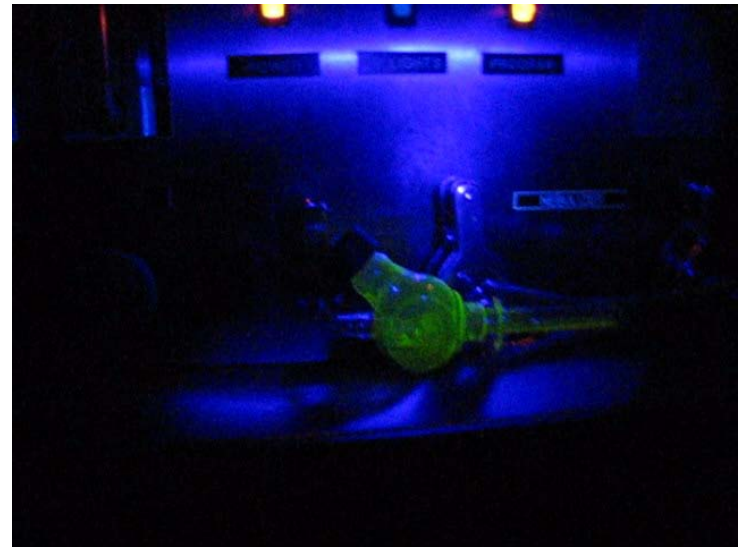
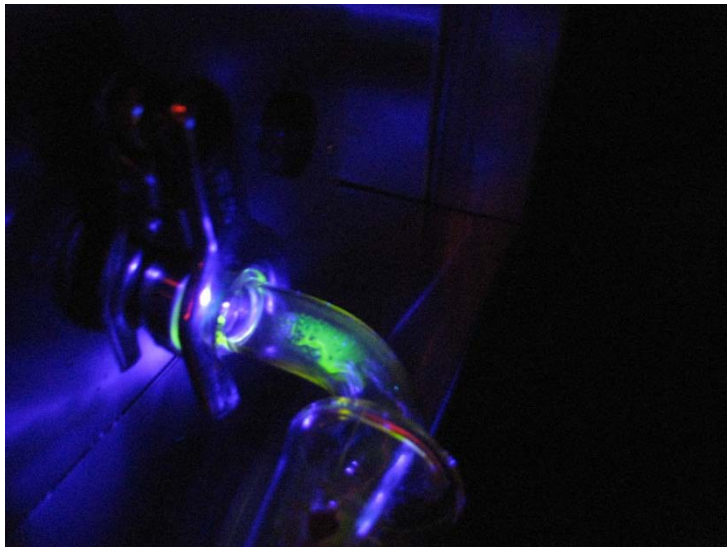
Experimental Design & Sampling Methods

- Fluorescein (sodium uranine)
 - 5 ml of 1% (w/v) in nebulizer
 - 50,000 μg total fluorescein
 - Surfaces are wiped down with distilled H_2O 2x prior to test
 - Visual inspection of surfaces with black light prior to test



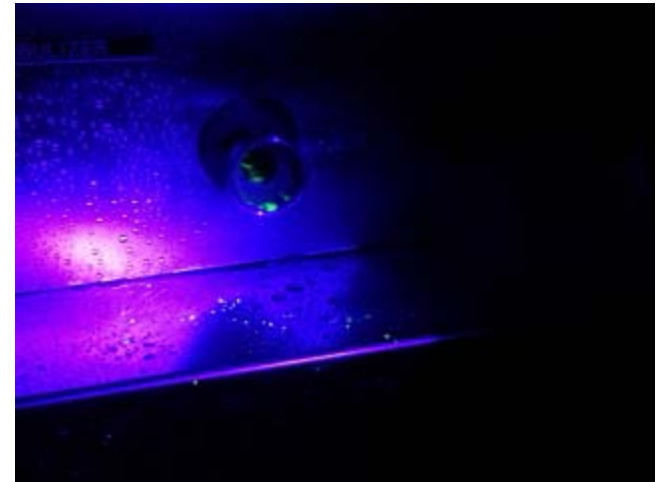
Experimental Design & Sampling Methods

- Standard operating program
 - Preheat: 15 min.
 - Nebulizing: 20 min.
 - Cloud decay: 20 min.
 - Decontamination: 15 min.
 - Cool Down: 10 min.



Experimental Design & Sampling Methods

- Qualitative results
 - Spray all surfaces with H₂O
 - Inspect with a black light
 - Note “leak” locations
- Quantitative results
 - Swab identified “leak”
 - Quantify amount of fluorescein in a fluorometer with standard curve



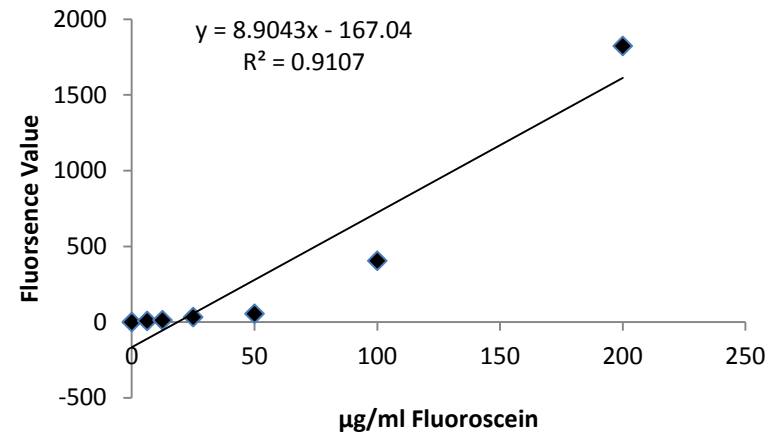
Sample Collection Efficiency

- Percent recovery of method:



- Foam swab = $\sim 0.77\%$
- Calcium Alginate = $\sim 97\%$

- Generate a standard curve for quantification:



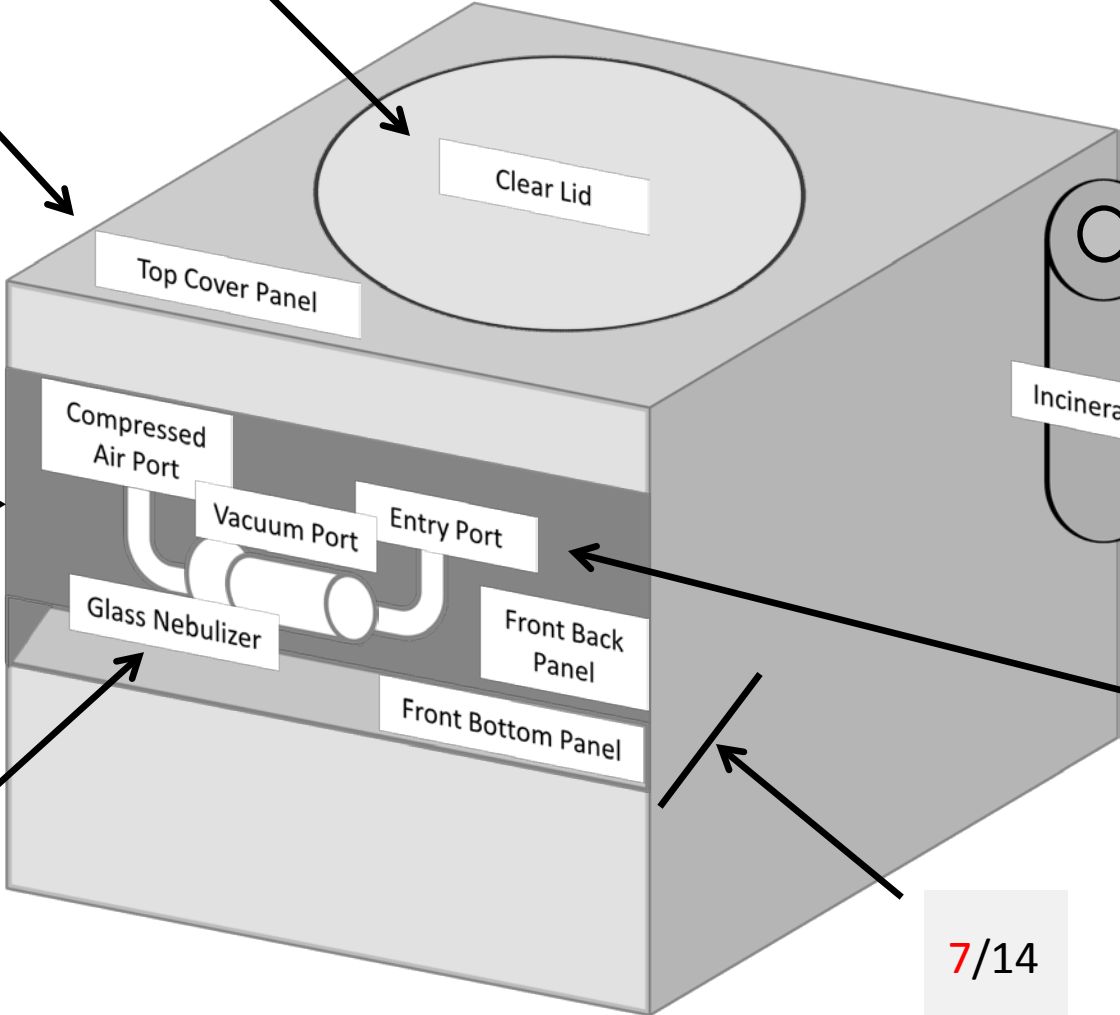
Standards	0	6.25	12.5	25	50	100	200
µg/ml	0.102	7.629	12.47	33.1	54.99	403.3	1822
	0.064	7.582	12.42	33.04	54.98	405.2	1822
	0.108	7.589	12.41	32.98	55.07	406.4	1827
Avg	0.091333333	7.6	12.43333	33.04	55.01333	404.9667	1823.667
Stdev	0.019482186	0.020704	0.026247	0.04899	0.040277	1.276279	2.357023

Total # of tests with ≥ 1 releases 7/14

4/14

0/14

0/14



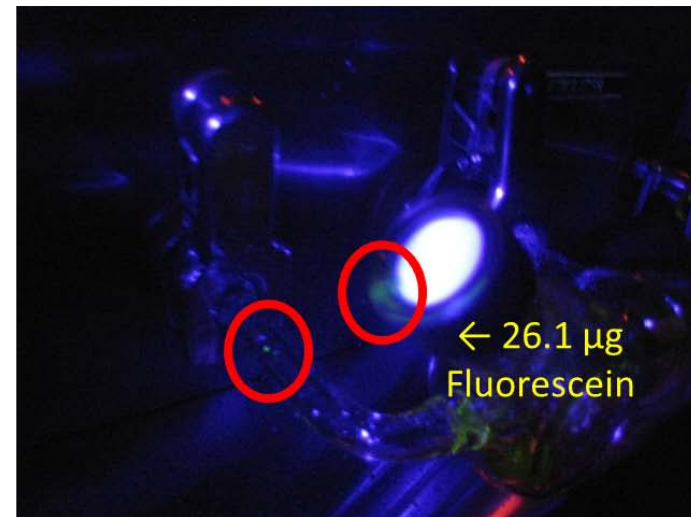
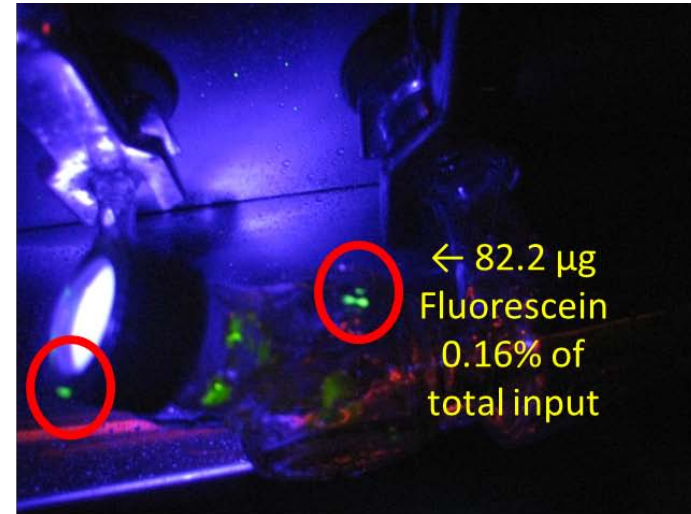
Pre-sample - Control
0/14

Post-sample + Control
14/14

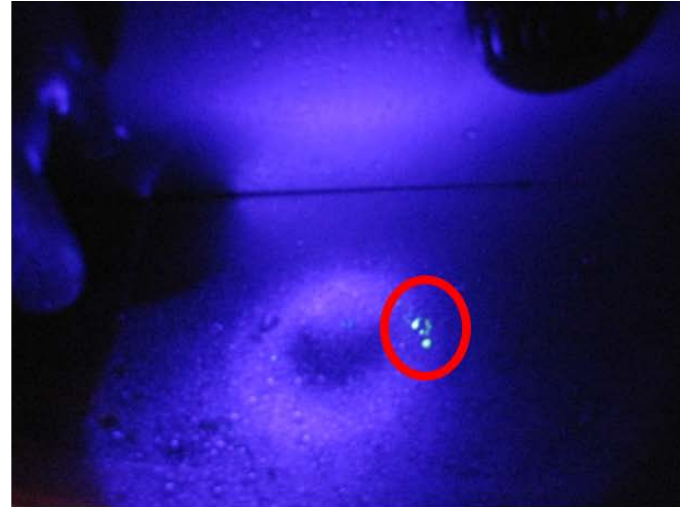
4/14

7/14

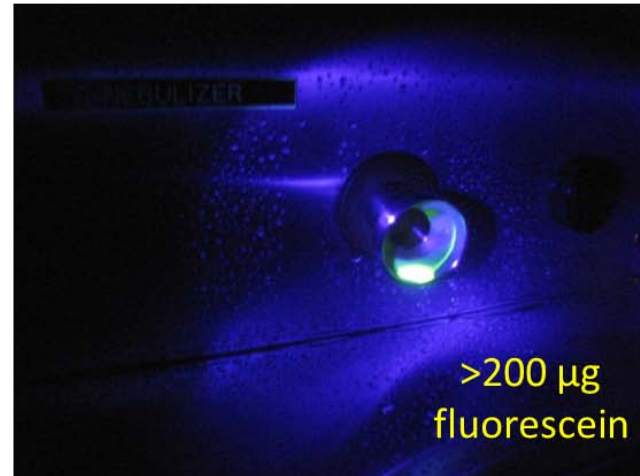
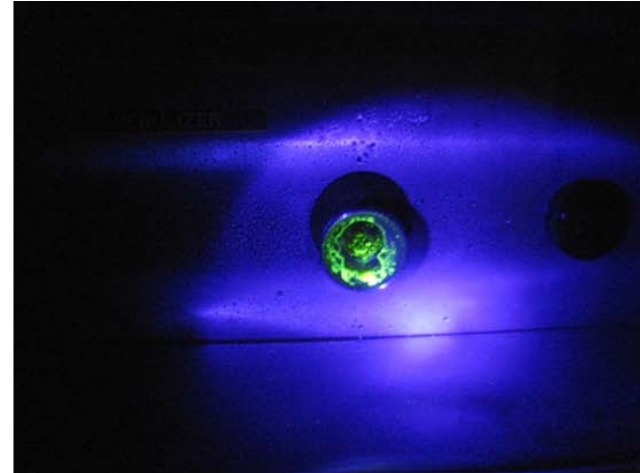
Fluorescein Release Examples- Glass Nebulizer



Fluorescein Release Examples- Areas Surrounding The Nebulizer



Post-Completion Residual Examples



Fluorescein Release During Cleaning



Summary

1. Fluorescein was detected outside of containment:
 - Nebulizer area positive for fluorescein **50%** of tests
 - Top cover positive for fluorescein **28%** of tests
 - Post-HEPA exhaust positive for fluorescein **0%** of tests

2. Fluorescein release identification could be conservative:
 - Samples limited to what can be visually identified
 - Aerosol particle lifetimes are expected to be high

3. Decontamination of system:
 - Post nebulizer port retains high amounts of fluorescein
 - Ensure effective decontaminating agents are used

Summary

4. System susceptibility to leaks likely depends upon:
 - User proficiency and experience
 - System maintenance and use

5. Fluorescein leak testing could be applied to numerous systems to generate data to support risk assessments

6. The following risk mitigation measures should be considered:
 - Respiratory protection
 - Tyvek coveralls
 - Double gloves
 - HEPA filtered BSL-3 laboratory
 - Personnel exit the laboratory before the aerosolization takes place and return only after it completes
 - Secondary sealing of openings (Ex. parafilm)
 - Secondary containment system

Acknowledgments

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