



Aerosol Monitoring of ABSL-4 Suites Housing Non-Human Primates Challenged with Ebola Virus

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Disclaimer

The views, opinions and findings contained herein are those of the author and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.



IACUC Disclaimer

Research was conducted under an IACUC approved protocol in compliance with the Animal Welfare Act, PHS Policy, and other Federal statutes and regulations relating to animals and experiments involving animals. The facility where this research was conducted is accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 2011.



Outline

- Background on external requirement primary containment caging for NHPs
- Proof of Concept/Purpose
- Methodology of viral RNA sampling and extraction
- Results of air sampling studies in animal rooms
- Recommendations/Findings



Background

- During 2011 inspection USAMRIID cited by CDC/DSAT for lack of primary containment caging system for NHPs in ABSL-4
- Other ABSL-4 laboratories within US received similar citations
- ABSL-4 suites housing infected animals require Form 3 submissions following mishaps within the animal room
- BMBL language on primary containment caging inconsistent
- Extensive debate between laboratories and regulators on requirement for primary containment caging





High Volume Aerosol Sampler

- Dry Filter Unit 1000 (DFU 1000) used for all animal room studies
- 100 cfm sampling rate
- Able to sample continuously for extended periods
- Durable and water resistant
- Utilized membrane (<math><1\mu\text{m}</math>) and standard $1\mu\text{m}$ filters to capture particulates



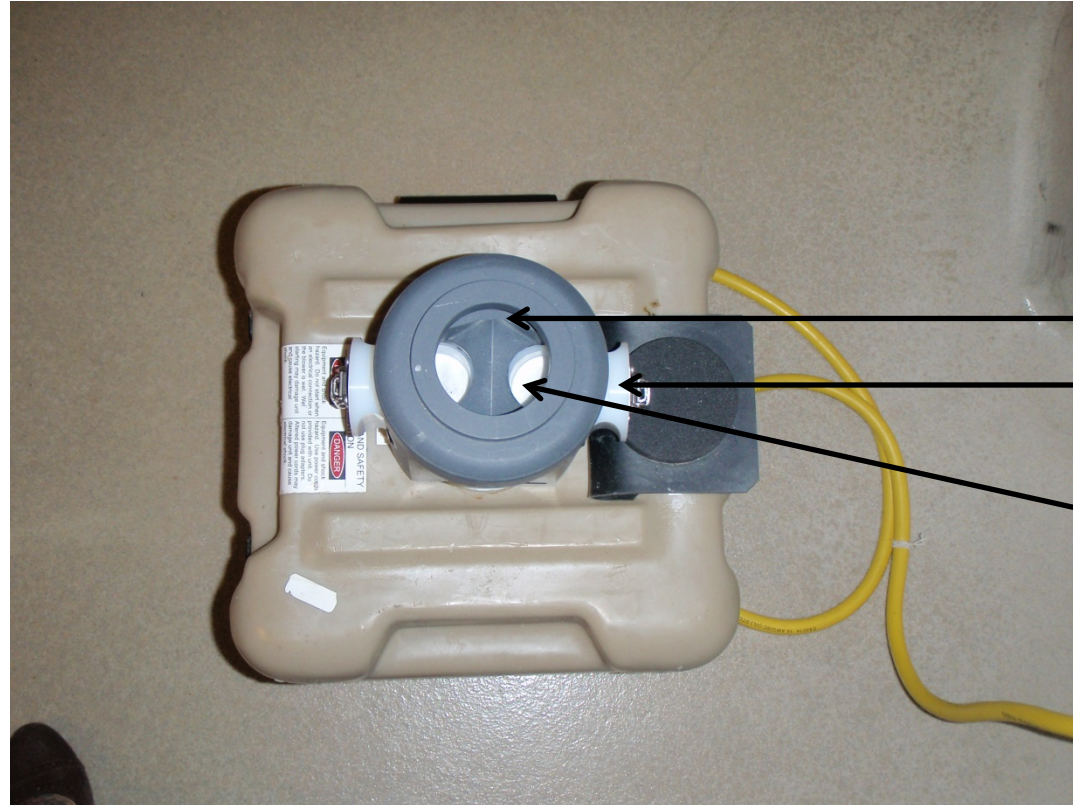
Air Inlet

Filter housing

Exhaust



High Volume Air Sampler (Top down view)



Air Inlet

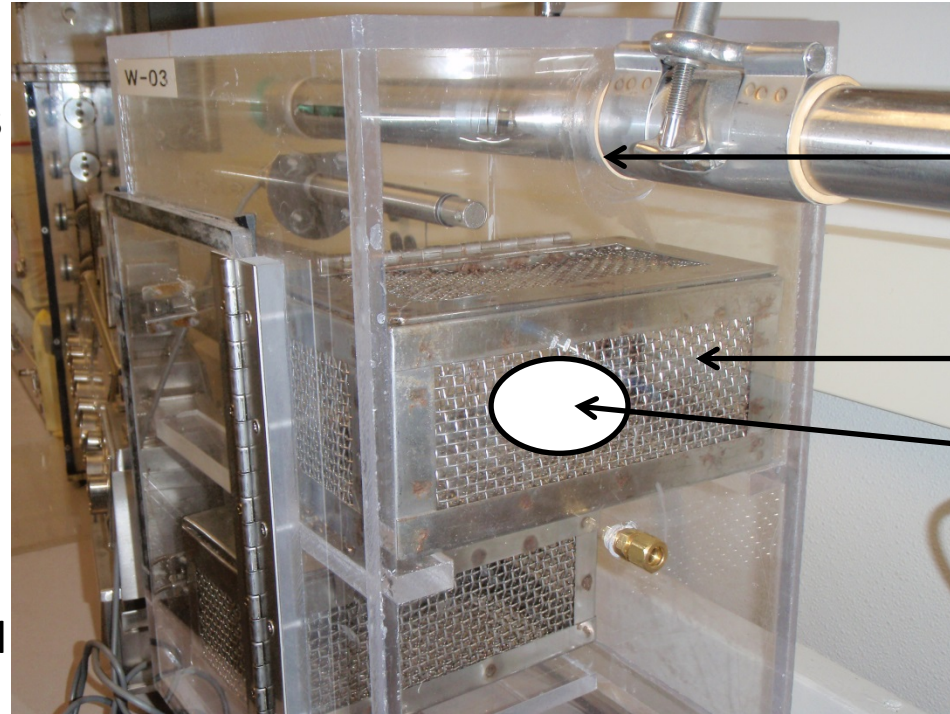
Filter housing

Filter



Proof of Concept Study

- Sham aerosol spray (no animals) conducted inside Class III BSC
- Ebola Sudan virus spray concentration ~200 pfu/mL
- Filters placed within exposure chamber to examine potential viral recovery
- Following spray filters were processed and RNA was extracted and inactivated prior to PCR analysis



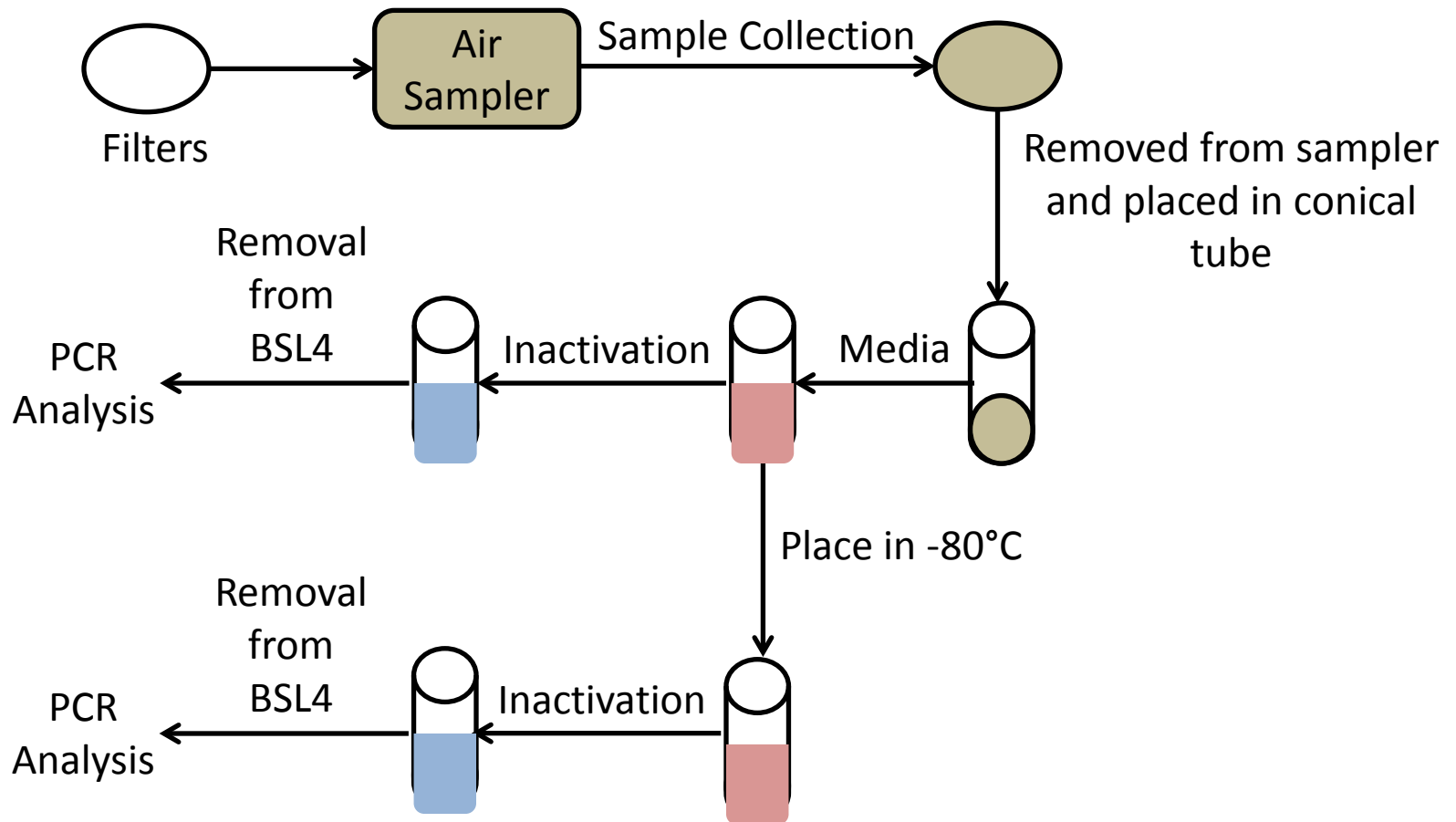
Collision tube
(Aerosol source)

Aerosol Chamber

Air Sampler
Filters



Filter Extraction Methodology



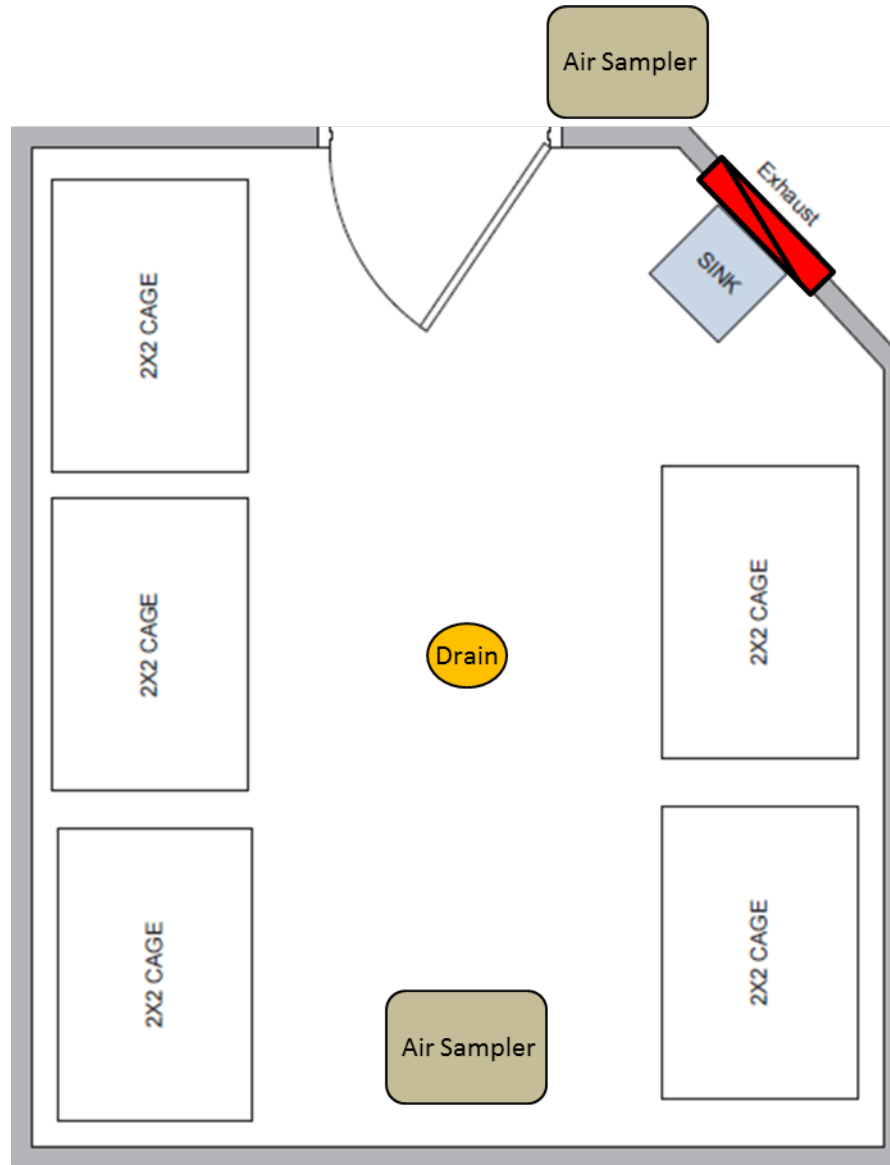


Animal Study Background

- Sampling conducted during three independent animal studies
- Studies were carried out in different ABSL4 suites by different research groups
- Sampling was carried out throughout the duration of each study(28 days)
- NHPs were challenged with Ebola Zaire strain through different routes (IM, aerosol)
- Research activities and husbandry were not restricted during sampling



Study 1 Setup





Study 1 NHP Outcomes

Route – IM challenge (leg)

Dose –1000 pfu/mL (Ebola Zaire R4415 stock)

NHP type – Rhesus macaque

Outcome (in terms of survival) – 12/20 NHPs survived challenges between controls and treatment groups

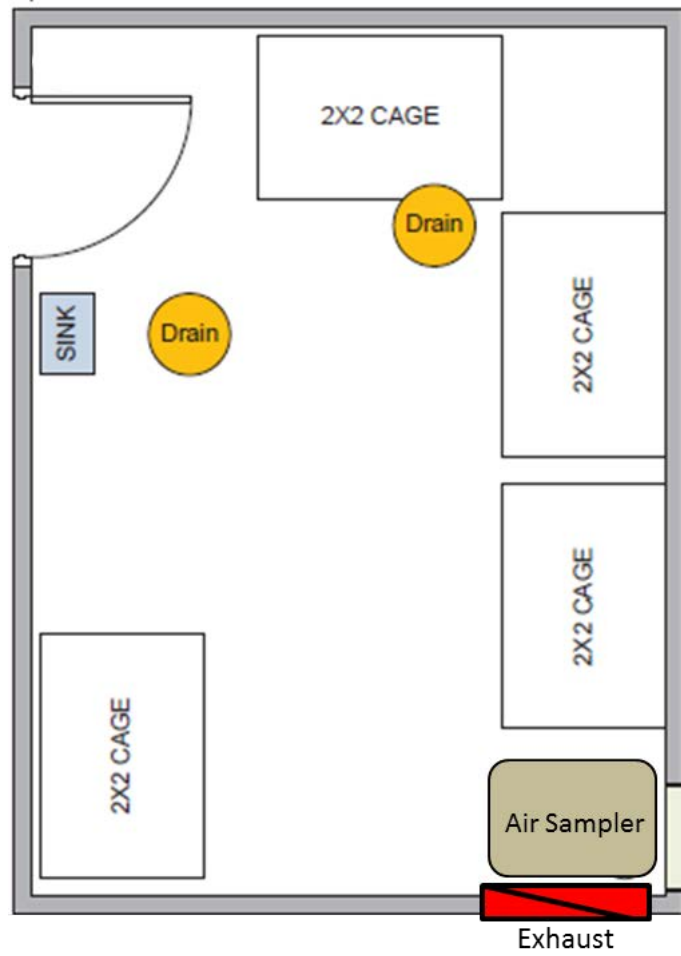


Study 1 Results

Day	Animal Room	Adjacent Suite Corridor
0	Negative	Negative
1	Negative	Negative
5	Negative	Negative
6	Negative	Negative
7	Negative	Negative
8	Negative	Negative
9	Positive	Negative
10	Positive	Negative
14	Negative	Negative
28	Negative	Negative
Positive Control 1	Positive	Positive
Positive Control 2	Positive	Positive
Negative Control	Negative	Negative



Study 2 Setup





Study 2 NHP Outcomes

Route –Aerosol/IM challenge

Dose –Ebola Zaire (R4415 stock ~200pfu)

NHP type – Cynomolgous macaques

Outcome (in terms of survival) – (3/12) NHPs survived challenges between controls and treatment groups

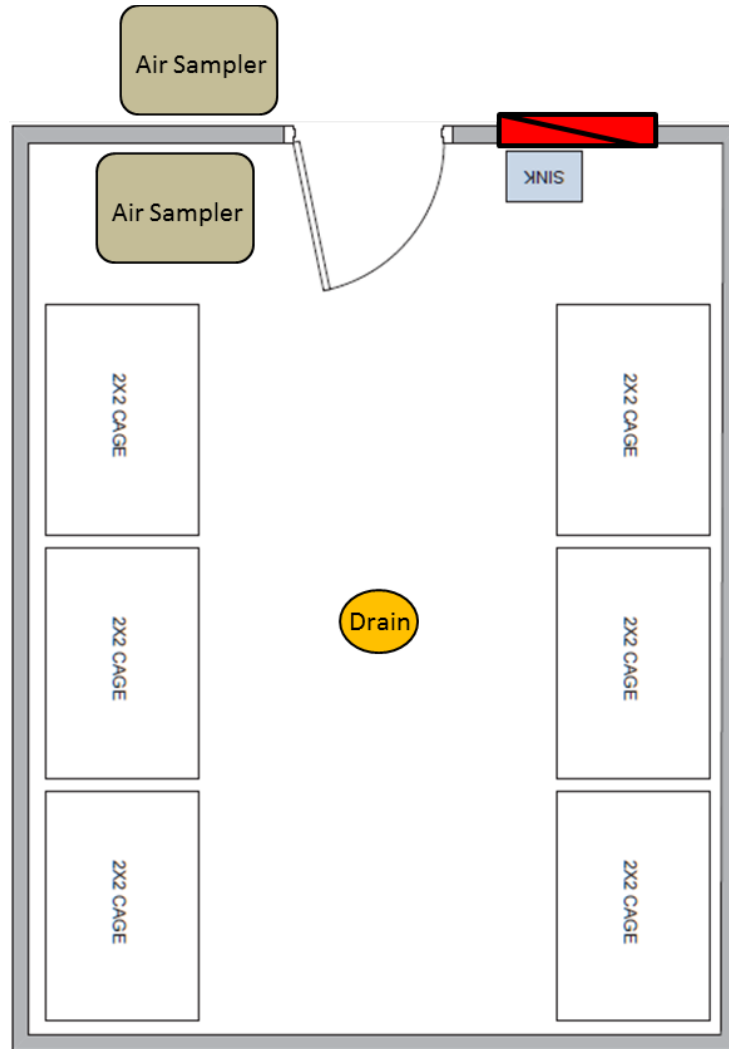


Study 2 Results

Day	Animal Room
0	Negative
1	Negative
5	Negative
6	Negative
7	Negative
8	Negative
9	Negative
10	Negative
14	Negative
28	Negative
Positive Control 1	Positive
Positive Control 2	Positive
Negative Control	Negative



Study 3 Setup





Study 3 NHP Outcomes

- Route – IM challenge (leg)
- Dose –Ebola Zaire (1170 pfu, R4415 stock)
- NHP type – Rhesus macaques

Outcome (in terms of survival) – 10/18 NHPs survived challenges between controls and treatment groups



Study 3 Results

Day	Animal Room	Adjacent Suite Corridor
0	Negative	Negative
1	Negative	Negative
5	Negative	Negative
6	Negative	Negative
7	Positive	Negative
8	Positive	Negative
9	Negative	Negative
10	Negative	Negative
14	Negative	Negative
28	Negative	Negative
Positive Control 1	Positive	Positive
Positive Control 2	Positive	Positive
Negative Control	Negative	Negative



Discussion

- PCR analysis conducted using specific markers for both Ebola Sudan (proof of concept) and Ebola Zaire (NHP challenges)
- Proof of concept demonstrated successful viral RNA recovery through PCR in each replicate
- Placement of high volume air samplers was limited due to space and safety considerations
- Membrane filters must be used in conjunction with standard filters to prevent degradation
- Significant debris recovered on filter media during animal studies



Discussion(continued)

- Positive PCR results obtained during peak viremic stages of infection in both Study 1 and Study 3
- No contamination was observed in hallways during either Study 1 or Study 3
- Hallway sampling was not conducted during Study 2 due to safety considerations
- While aerosolization of EBOV RNA may be observed during peak periods of infection it is an infrequent occurrence
- Lack of hallway contamination demonstrates success of administrative controls, PPE and training during NHP studies



Conclusions/Recommendations

- Ebola RNA can be shed by NHPs during the death window stages of infection
- Potential presence of aerosolized Ebola RNA occurs infrequently and is study dependent
- PPE and primary containment recommendations need to be evaluated on a case by case basis
- Due to the **absence** of Ebola RNA within the hallway the risk of utilizing primary containment caging systems does not outweigh the perceived benefit within the ABSL-4 environment since current administrative and engineering controls function properly to protect personnel and the environment from potential contamination



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Questions?