Performing Inhalation Exposures Using Infectious Microorganisms and Toxins: Conducting Them Safely, Accurately, and Reproducibly.

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Vaccines and pharmaceuticals that are used to protect against or treat diseases caused by biological terrorist agents (bacteria, viruses and toxins) are often evaluated for efficacy via inhalation, which is a probable route of exposure from an attack or natural infection. Models of disease need to be developed using well characterized inhalation systems using highly controlled procedures and processes in order to ensure, safety, reproducibility and consistency in the inhaled or presented dose. This is especially true for research which will be used in support of licensure by the U.S. Food and Drug Administration or other licensing entity. This presentation will briefly outline the universal components of inhalation exposure systems, considerations on the model to be tested, reproducibility, methods to quantify the agent dose, safety aspects of conducting studies within the limited confines of a Biosafety Cabinet Class III (BSC-III), and data examples for dose accuracy and reproducibility will be discussed.

Constructing inhalation systems and performing inhalation studies are complex with many challenges. An inhalation exposure system for dosing/challenging consists of four or five subsystems; an aerosol generator, delivery device, an exposure chamber/atmosphere, exposure sampling/monitoring device(s), and a method for determining respiratory parameters for dose determination or plethysmography. The methodology for dose quantification will be microorganism dependent with the spread plate method primarily used for bacteria and plaque assay for viruses.

Figure 1 below shows the limited space that is available to construct an inhalation exposure system and conduct inhalation studies.

Figure 1. Photograph showing the limited space in BSCIII

Process, procedures, and controls (administrative and engineering controls) must be implemented to ensure the safety of the staff conducting the studies to prevent accidental infection and possibly death. Once these are addressed, an inhalation study may commence.

Table 1 demonstrates data showing accuracy and reproducibility of mean and standard deviations for dose delivery that are within 15% or better of the targeted dose (good data) compared to data showing dose delivery between 195-584% of the targeted dose (poor data). Figure 2 shows reproducible mean daily doses for three dose concentrations across 15 test days for *B. anthracis* spores.

Table 1. Comparison of targeted dose to actual dose for two studies

Target Dose (LD_{50}s)	Mean (LD_{50}s)	Standard Deviation (LD_{50}s)	Range (LD_{50}s)	
			Low	High
200	173	57	114	332
200	175	56	120	297
200	201	76	127	362
200	173	25	120	222
200	178	34	139	239
80	467	379	NP (~1)	NP (~1298)
80	156	97	NP (~1)	NP (~359)
80	269	258	NP (~1)	NP (~809)

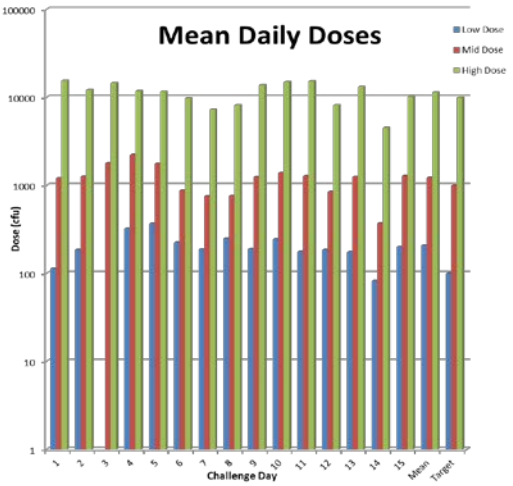

Figure 2. Comparison of consistent doses over fifteen test days. (Barnewall et al., 2012)

The Inhalation challenge is a complex process with many factors to consider to conduct safely and reproducibly.

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