Aerosol Characterization of Three *Bacillus anthracis* Spore Preparations

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**Abstract**

*Bacillus anthracis* (B. anthracis) is the causative agent of cutaneous, gastrointestinal, and inhalational anthrax. In inhalational anthrax models, *B. anthracis* is induced to form spores that are used to challenge animals. Since many different *B. anthracis* spore preparation protocols exist that produce different aerosol particle size distributions, we characterized aerosol from different *B. anthracis* Sterne (BAS) purification processes to determine which process produces the best type of spore preparation. Our parameters for the ideal spore preparation were: 1) the most consistent and 2) the highest percentage of particles with the appropriate size (1 to 2 microns). We hypothesized that renograin purified spores would give us the most consistent results and analyzed three different purification processes: washed, pellet, and renograin purified. The mean aerosol concentration (cfu/L) and impinger concentrations (cfu/mL) were higher in the renograin preparations compared to washed or pellet purified spores. However, only ~13% of the washed spores had a count median diameter between 1 to 2 microns. The impinger concentrations (cfu/mL) were higher in the renograin samples compared to washed or pellet purified. The renograin preparation demonstrated approximately 90% of the count median diameter between 1 to 2 microns. We conclude that renograin purification of BAS spores is necessary to obtain clean and consistent bioaerosols for use in inhalation anthrax models.

**Methods**

**Preparations of bioaerosols:** BAS was grown in Difco Sporulation Media for 48 hours and harvested by centrifugation. Then washed, pellet, and renograin purification were done. Following each purification all spore preparations were resuspended in USP water, plate counted in triplicate, and diluted to 1×10^6 CFU/mL. Spore bioaerosols were generated with a Collison 3-jet nebulizer and collected from a plenum with an all-plastic impinger (AGI) containing USP water. Relative AGI concentrations of spores were determined by additional plate counting.

**Wash purification:** The spores were washed a total of three times with USP water.

**Pellet purification:** The spores underwent a process in which the outer layer of the spore pellet was removed by gently swirling and pipetting to remove the loosely pellet material.

**Renograin purification:** The spores were passed though a 20:30% (v/v) renograin gradient.

Five different spore preparations were analyzed. Particle counts and size were taken for each preparation. Mean t-tests were performed to determine statistical significant for impinger and aerosol concentrations.

**Results**

Figure 1. Representative sample of a spore pellet debris removed in the pellet purification process. A large amount of total cell volume is lost in the pellet purification process, which could lead to its variability between preparations.

Figure 2. A representative sample of two separate preparations of spores. A) Renograin purified spores, B) Pellet purified spores, and C) Washed spores. A visual comparison of the purification process shows variability between preparations.

**Rodent nose-only aerosol inhalation system**

Figure 3. Rodent nose-only aerosol inhalation system. A) ITP 24-port chamber, B) Stainless steel impinger, C) DustTrak (particle counter), and D) Aerodynamic particle sizer.

Figure 4. The mean concentration of the different spore preparations collected from the AGI and calculated aerosol concentrations. The mean aerosol concentration (cfu/L) and impinger concentration (cfu/mL) were higher in the renograin purified spore preparations compared to washed or pellet, but were not significant.

**Particle number comparison of wash, pellet, and renograin purified spores**

Figure 5. MicroDust comparison of two data sets. The aerosol particle concentration varies in both the wash and pellet purified samples; whereas, the renograin purified samples are reliably consistent.

Figure 6. Size distribution of spores from each type of preparation. The mean count median values for washed, pellet, and renograin purified spores were 0.79, 1.03, and 1.26 respectively and statistically significant. The renograin purified spores had the most particulates between 1 micron and 2 microns compared to the washed and pellet purified spores.

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