**Supplementary information**

**for**

**Toward Biological Aerosol Reference Standards**

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**BA Sterne Spore Protocol**

Project: Prediction of Aerosol Viability from Exposure on Surfaces (PAVES)

Task: Bacillus anthracis Sterne Spore Preparation- Set #1

**Timeline and Steps:**

7/3/17- a plate of Bacillus anthracis Sterne was struck from frozen culture collection (Lot# 032607). Plates were left at RT for 48 hours due to holiday.

7/5/17- Growth was observed, with single colonies present. Plates were stored at 4°C in Lab 307fridge. Two 20 mL TSB cultures of Ba Sterne were started prior to storage.

7/7/17- Aliquoted 400 μl of the O/N/G broth culture and spread on to 4 Lab Lemko plates. (10 plates remain of same prep).

7/13/17- spores from sporulation set #1 were checked to assess number of vegetative cells vs. spores (microscope to analyze wet mounts). Culture seeded was at-90%. Spores will be harvested tomorrow.

7/14/17- Harvested spores off the 4 plates from sporulation set # 1; Washed 2 plates each with 15 ml of sterile water; Rewashed 4 plates total with 15 ml of sterile water before washing all with a third 10 ml
Therefore to harvest all the plates, 55 ml sterile water was used. The spores harvested from the plates were filtered through an autoclaved paper towel; The harvested suspension was centrifuged for 2 hours. Clear supernatant was aspirated and the pellet was washed with 40 ml of sterile water. The suspension was centrifuged again for 1 hour. The clear supernatant was aspirated and the pellet washed with 40 mL 0.1 % Tween 80. The suspension was centrifuged again, and the supernatant aspirated; The pellet was resuspended in 30 ml 0.1 % Tween 80; Suspension was stored at 4 °C for the weekend prior to being heat shocked due to water bath not being at the appropriate level.

7/18/2017 Sporulation set #1 suspension heat shocked at 65°C for 30 minutes to eliminate any remaining vegetative cells. Stock was also sonicated for 5 minutes and vortexed for 2 to break up any clumps of spores then returned to 4 °C. Prior to storage, the stock culture was enumerated. Spores analyzed under the microscope looked >95% with no vegetative cell burden

7/19/2017 The enumeration plates were counted and the stock titer averaged: 3.6x109/mL .A working stock was made by adding 5ml of 3.6E9 parent stock to 95 ml of 0.1% Tween 80; the working stock was divided between two tubes in which 2.5 mL of parents stock was added to 4 - 7.5 mL T-80 each. The working stock was enumerated.

7/20/2017 Enumeration plates were counted and the average l .25x 108 /mL. This working stock labeled as 1.25E8.