



AEROSOL GENERATION AND AEROSOL PARTICLE CORRELATIONS ON TRIALS CONDUCTED WITH SONO-TEK ULTRASONIC NOZZLE IN JOINT AMBIENT BREEZE TUNNEL

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Introduction

Biological aerosols of *Bacillus thuringiensis* Kurstaki (BTK) were generated with the Sono-Tek spray nozzle system in the Joint Ambient Breeze Tunnel located in Dugway, Utah, for the first time. Aerosol particle concentration and size distribution were monitored with the Aerodynamic Particle Sizer® (APS). Test results demonstrated that BTK aerosols could be generated by using Sono-Tek aerosol generator in a controllable way to generate aerosol with realistic concentrations and particle size distributions. We found that aerosol mass concentration vs. BTK total feed (volume of BTK slurry atomized) has better correlation than aerosol number concentration vs. BTK total feed due to the inherent high concentration of polydisperse natural background particles in the JABT during the trials. It is more appropriate to use mass concentrations instead of number concentrations to correlate with sample concentrations [genome equivalent (GE)/mL and colony-forming unit (CFU)/mL].

Methods

Joint Ambient Breeze Tunnel (JABT) (**Figure 1.left and right**) was built to test various integrated platforms: System-Under-Test Units (SUTs) simultaneously. The JABT is 168 meters in total length, 13 meters in width, and 18 meters in height, with an adjustable canopy/ceiling to adjust the height of the test space. The airflow velocity in the JABT is independent of outside wind condition and can be maintained in a range of 0 to 6 meters per second. Similar particle size distributions (multi-modes and polydisperse) and concentrations can be generated in the JABT, as at a field test grid, though fewer materials are used in the JABT due to its controlled environment. Trials were conducted 10-22 May 2023 to demonstrate simulant aerosols could be generated using a Sono-Tek ultrasonic atomizer® (Sono-Tek. Model No. 06–04004, Sono-Tek Corporation, Milton, NY) in a controllable way with realistic aerosol concentrations and particle size distributions.

Bacillus thuringiensis Kurstaki (BTK) is a common simulant for *Bacillus anthracis* for biodefense research. A BTK slurry suspension (6.95×10^6 CFU/mL) of BTK powder into 1 L of distilled water) was fed into the Sono-Tek nozzle using a syringe pump. When necessary, two sets of aerosol generation systems were used to achieve the total feed needed. A Broadband Ultrasonic Generator (BUG, (Model No. 06-05108) generated high-frequency vibrations to disrupt the slurry flow at the Sono-Tek nozzle tip. The vibrations atomized the slurry into an aerosol cloud carried by the airflow to the down airstream detection and collection area containing one MD8 Airscan Air Sampler® (MD8, Sartorius Stedim, North America Inc, Bohemia, NY) and one Aerodynamic Particle Sizer® (APS, TSI Incorporated, Shoreview, Minnesota) in JABT (**Figure 1. right**). A Sono-Tek a BUG) with a programmable syringe pump; Sono-Tek Corporation, Milton, NY) allowed for the discrete control of the injection rate of aerosol mass into the JABT.

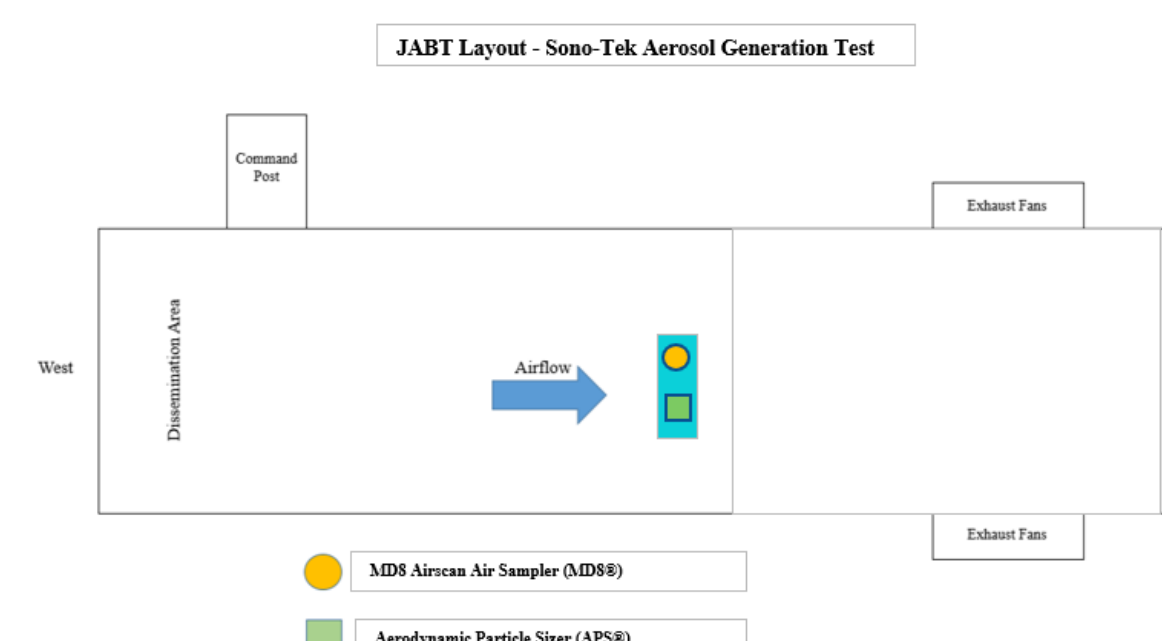


Figure 1. left. JABT outside view from the east; **right.** Layout of Sono-Tek Test.

The dry particle size of the aerosols generated by the Sono-Tek was controlled by the concentration of the slurry in the liquid being atomized. Aerosol particle size and concentration were measured by one APS (using a particle size range from 0.3 µm to 20 µm aerodynamic diameter. An air sampling rate for the MD8 was set for 120 L/Min through its gelatin filter for each trial. Each gelatin filter was collected and dissolved in 10 mL sampling solution for colony-forming unit (CFU) assays and a polymerase chain reaction (PCR) analysis using an Applied Biosystems QuantStudio 12K Flex Real-Time PCR System.

Results

By varying the feed rate (mL/Min) and duration (Min) of slurry injection, the amount of material generated (mL) [Sono-Tek total Feed (mL) in **Table 1**] can be modified and used to control the concentration of material introduced into the JABT. Summary of average aerosol number and mass concentrations, liquid sample number, mass, CFU, PCR concentrations, and Sono-Tek total feed (mL) were shown in **Table 1**. We used APS as referee equipment to acquire necessary aerosol data and MD8 as aerosol collector to capture aerosol particles in gelatin filter before taking the samples to an indoor laboratory to be analyzed by RT-PCR to determine sample PCR concentration (2 and 3GE/mL) and by plate count to determine sample CFU concentration (CFU/mL). A few relationship equations shown in **Figure 2** have been formulated based on data in **Table 1**.

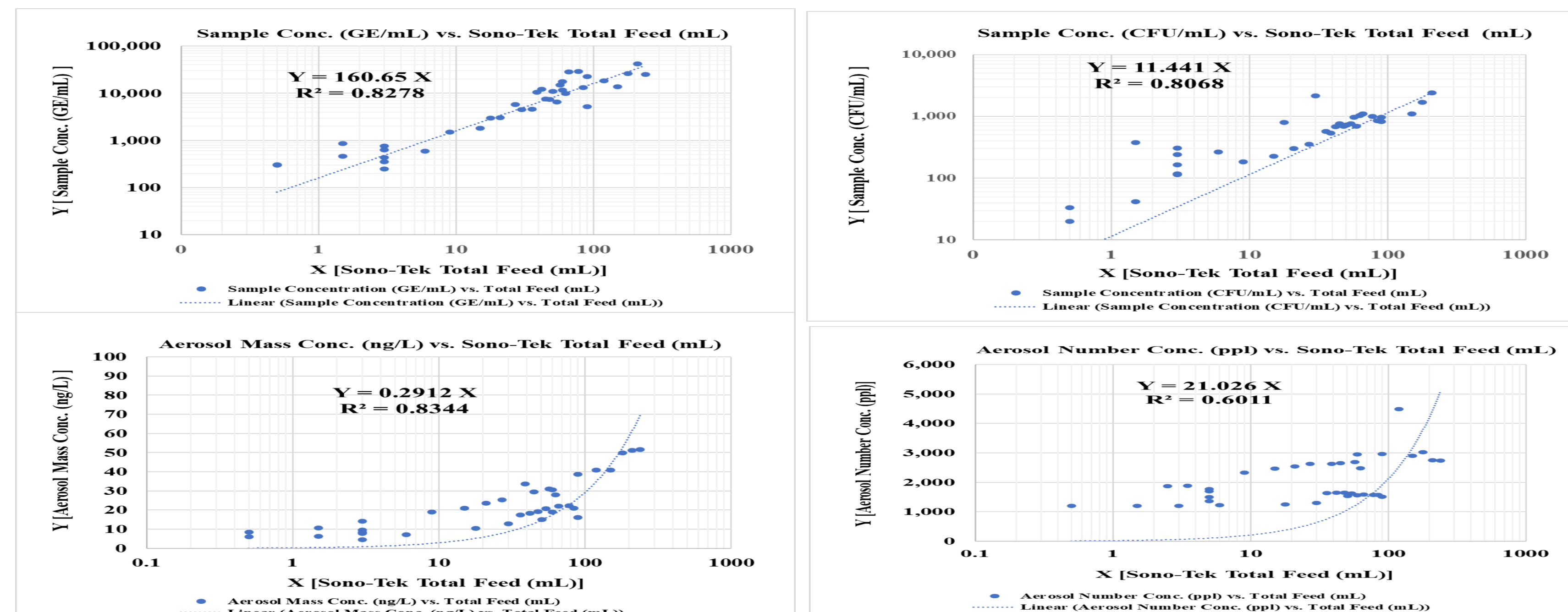


Figure 2. Top left. Relationship of Sample Concentration (GE/mL) on Log₁₀ Scale vs. Sono-tek Total Feed (mL) on Log₁₀ Scale; **Top right.** Relationship of Sample Concentration (CFU/mL) on Log₁₀ Scale vs. Sono-tek Total Feed (mL) on Log₁₀ Scale; **Bottom left.** Relationship of Aerosol Mass Concentration (ng/L) vs. Sono-Tek Total Feed (mL) on Log₁₀ Scale; **Bottom right.** Relationship of Aerosol Number Concentration (ppb) vs. vs. Sono-Tek Total Feed (mL) on Log₁₀ Scale.

Table 1. Summary of Average Aerosol Number and Mass Concentrations, Liquid Sample Number, Mass, CFU, PCR Concentrations, and Sono-Tek Total Feed (mL).

Trial # BT-	N ₁ ¹ (n/L)	M ₁ ² (ng/L)	Sample Number Conc. ³ (n/mL)	Sample Mass Conc. ⁴ (pg/mL)	Sample Conc. (CFU/mL)	Sample PCR Conc. (GE/mL)	Sono-Tek Total Feed ⁵ (mL)
PreT-003	2.74E+03	51.52	3.29E+05	6.18E+06	-	2.51E+04	240
PreT-004	4.49E+03	40.79	5.38E+05	4.89E+06	-	1.85E+04	120
PreT-005	2.94E+03	30.7	3.53E+05	3.68E+06	-	1.75E+04	60
T-001	1.30E+03	12.78	1.55E+05	1.53E+06	2.15E+03	4.55E+03	30
T-002	1.25E+03	10.39	1.50E+05	1.25E+06	8.00E+02	2.95E+03	18
T-003	1.22E+03	7.08	1.47E+05	8.50E+05	2.65E+02	5.93E+02	6
T-004	1.21E+03	7.74	1.45E+05	9.29E+05	1.17E+02	7.63E+02	3
T-005	1.20E+03	6.26	1.01E+05	5.26E+05	4.17E+01	8.65E+02	1.5
T-006	1.20E+03	6.17	7.20E+04	3.70E+05	2.00E+01	2.98E+02	0.5
T-007	1.63E+03	17.38	1.95E+05	2.09E+06	5.73E+02	4.59E+03	36
T-008	1.65E+03	18.41	1.98E+05	2.21E+06	6.80E+02	1.22E+04	42
T-009	1.65E+03	19.18	1.98E+05	2.30E+06	6.87E+02	7.41E+03	48
T-010	1.62E+03	20.82	1.94E+05	2.50E+06	7.57E+02	6.53E+03	54
T-011	1.59E+03	22.02	1.90E+05	2.64E+06	1.10E+03	2.83E+04	66
T-012	1.57E+03	18.94	1.50E+05	1.82E+06	6.90E+02	1.17E+04	60
T-013	1.57E+03	22.26	1.88E+05	2.67E+06	1.00E+03	2.92E+04	78
T-014	1.58E+03	20.96	1.89E+05	2.52E+06	8.53E+02	1.31E+04	84
T-015	1.51E+03	16.17	1.81E+05	1.94E+06	8.23E+02	2.24E+04	90
T-016	1.54E+03	15.01	1.84E+05	1.80E+06	7.30E+02	1.09E+04	51
T-017	2.96E+03	38.71	3.55E+05	4.65E+06	9.60E+02	5.24E+03	90
T-018	2.90E+03	40.80	3.48E+05	4.90E+06	1.10E+03	1.38E+04	150
T-019	3.02E+03	49.95	3.63E+05	5.99E+06	1.70E+03	2.61E+04	180
T-020	2.63E+03	25.37	3.15E+05	3.04E+06	3.53E+02	5.74E+03	27
T-021	2.54E+03	23.51	3.05E+05	2.82E+06	3.03E+02	3.05E+03	21
T-022	2.47E+03	20.88	2.96E+05	2.51E+06	2.27E+02	1.81E+03	15
T-023	2.33E+03	19.05	2.80E+05	2.29E+06	1.83E+02	1.52E+03	9
T-024	2.75E+03	51.28	3.30E+05	6.15E+06	2.40E+03	4.21E+04	210
T-025	2.63E+03	33.77	3.15E+05	4.05E+06	5.37E+02	1.05E+04	39
T-026	2.65E+03	29.44	3.18E+05	3.53E+06	7.67E+02	7.58E+03	45
T-027	2.68E+03	30.99	3.22E+05	3.72E+06	9.63E+02	1.49E+04	57
T-028	2.48E+03	27.94	2.98E+05	3.35E+06	1.02E+03	9.91E+03	63
T-029	1.37E+03	4.59	1.64E+05	5.51E+05	1.13E+02	2.47E+02	3
T-030	1.50E+03	9.55	1.80E+05	1.15E+06	3.07E+02	3.51E+02	3
T-031	1.77E+03	14.07	2.13E+05	1.69E+06	1.63E+02	4.39E+02	3
T-032	1.70E+03	8.56	2.03E+05	1.03E+06	2.40E+02	6.35E+02	3
T-033	1.88E+03	10.57	1.58E+05	8.88E+05	3.77E+02	4.61E+02	1.5
T-035	1.87E+03	8.39	1.12E+05	5.03E+05	3.33E+01	3.06E+02	0.5

Note: Challenging material – *Bacillus thuringiensis* Kurstaki (**BTK**); Average Aerosol Number Concentrations N₁¹ [average size range: 0.898 – 20 µm during APS collection time, in unit of n/L, or Particles per liter of air (ppl)]; Average Aerosol Mass Concentrations M₁² [average size range: 0.898 – 20 µm during APS collection time, in unit of ng/L]; Sample Number Conc.³ (n/mL) = [Collection Time (Min) × (120 L/Min) × (N₁(n/L))/10 mL; Sample Mass Conc.⁴ (pg/mL) = [Collection Time (Min) × (120 L/Min) × M₁(ng/L)](1000pg/ng)/10 mL; ^a- Not analyzed; Sono-Tek Total Feed⁵ = feed rate (mL/Min) × duration (Min) of slurry injection; APS data of T-034 is not recorded, not included in the table.

Conclusions

We found that aerosol mass concentration vs. BTK total feed (volume of BTK slurry atomized) has better correlation than aerosol number concentration versus BTK total feed due to the inherent high concentration of polydisperse background particles in the JABT. It is more appropriate to use mass concentrations instead of number concentrations to correlate with sample concentrations (GE)/mL and colony-forming unit (CFU)/mL. This study represented the first time the Sono-Tek aerosol generation system has been used in the JABT. Test results demonstrated controlled aerosol could be generated to provide realistic concentrations and particle size distributions. The results of this study also support the use of the Sono-Tek aerosol generation system for future testing if more precise control of aerosol generation is required to challenge SUTs at low aerosol concentrations.

Acknowledgements: The authors express their appreciation to the following individuals for their guidance: Dr. Jeff Hogan of BioTesting Division management; and Mr. Damon Nicholson of Special Program Division. Appreciation is extended to Dr. John M. Hurley, Mr. Jonathon M. Hogan, Mr. Jeffery Poor, and other employees of BioTesting Division who supported us in our study.



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