



Each organism has a specific protocol for culture preparation. Each freeze-dried culture obtained from American Type Culture Collections (ATCC) was reconstituted and grown according to their protocol for the initial culture. Subsequent cultures were optimized to produce the desired organism titer. Each organism has a transmittance: log₁₀ viable organisms/ml calibration curve developed using spectrophotometer readings and serial dilutions. This allows each experiment's nebulization to have approximately the same organism titer. Table 1 has information regarding the various organisms agreed upon by Dust Free and Universal Air Technology.

Table 1 Microorganism Culture Overview

Organism	ATCC #	Growth Media	Growth Temperature (°C)	Approximate Incubation Time (hours)
<i>Serratia marcescens</i> (Gram negative bacteria)	14756	Plate Count Agar	30	20-24

Culture preparation and cell suspension for *Serratia marcescens* involves:

1. Inoculate multiple 3-ml broth test tubes.
2. After 20-24 hour incubation at 30°C,
3. 1.7-ml micro-centrifuge tubes are filled with the culture.
4. Centrifuge micro-centrifuge tubes for five minutes at 6000 rpm.
5. The supernant is removed, and the pellet is resuspended in 1.2-ml sterile deionized water.
6. The suspension is allowed to sit for 30 minutes to allow the larger cellular particulate to migrate towards the bottom of the tube (this step can be replaced by a short centrifugation).
7. The top cell suspension layer (600 - 800 µls) from each microcentrifuge tube pair is transferred into a new microcentrifuge tube.
8. This suspension can then be diluted for nebulization, or used in the optical density calculations. If further washings are required, steps 4-7 are repeated.

For each suspension prepared, an optimum colony forming unit (CFU) concentration was established. Universal Air Technology has a BGI three-jet MRE-type Collision Nebulizer. This nebulizer was used to generate target concentrations between 200 and 300 CFU/ft³. The duct used for these bioaerosol tests is a 2.75 SQFT duct with 300 FPM, and 825 CFM flow through the duct. 8.93E+07 CFU/ml suspensions were used for *Serratia* bioaerosol tests.

UV Single Pass Bioaerosol Efficiency Sample Protocol

Back to Back Samples (*Serratia marcescens*):

1. Close any open door or valves on duct.
2. Be sure both samplers are plugged into electronic timer/controller and are switched on.
3. Prepare culture, install nebulizer, and close duct.
4. Turn on air handler and allow one minute for stabilization.
5. Begin nebulization, and allow one minute prior to first sample.
6. Position agar plate on bottom Anderson sampler stage.
7. Attach sampler to appropriate sampling port upstream of the nebulizer.
8. Repeat step 5 for downstream port.
9. Samples are for one min, (1 CFM), open valve for sampling ports in use.
10. Turn on timer/controller.
11. After sampling time, close sampling port valves.
12. Remove samplers, remove and replace agar plates, reposition sampler, and reset timer.
13. Turn UV system ON (or Off, alternating between two settings)
14. Start timer/controller.
15. Continue steps 11-14 until ten sample sets with UV ON and ten sample sets with UV OFF are collected.
16. Turn off nebulizer, and allow air handler to run for five additional minutes prior to opening duct (clear air stream).