

Universal Air Technology, Inc. 2208 NW 71st Place, Suite B Gainesville, FL 32653 (352) 379-9630

May 14, 2001

RE: PO ID# Q03131

Mr. Gregg Burnett Dust Free, Inc. 1112 Industrial Park Drive P.O. Box 519 Royse City, TX 75189

Dear Gregg Burnett:

Subject: Results from Research and Development Testing

Test data from the five UV light systems specified in your purchase order are included in the attached computer files. Two of the three organisms were tested with the specified lights and only data from these two organisms is included. The attached files include: a file with general procedures used for the bioaerosol tests, a file with the data collected for the *Serratia marcescens* bioaerosol tests, a file with the data collected for the *Serratia marcescens* bioaerosol tests, a file with the data summaries for both organisms. In addition, the data summary files were added to the following two pages.

We hope you find this information useful. Please contact UAT if you need further clarification regarding the information provided. Thank you.

Best regards, Henry Greist

Microbiologist Universal Air Technology, Inc. Computer File Attachments (4)



Table 1: Aspergillus niger UV Performance

Aspergillus niger

UV Light Comparison

	-		Light Off (cfu/cu.ft.)				Light On (cfu/cu.ft.)			
		% Kill after		After				After		
	Date	Background		Diffusion		%		Diffusion		%
	Completed	Subtracted	Before unit	Plate	Reduction	Reduction	Before unit	Plate	Reduction	Reduction
Healthy Climate (TM) UV-2000	04/28/01	2.462	219.9	178.6	41.3	-18.781	210.8	176.4	34.4	-16.319
Healthy Climate (TM) UV-1000	04/28/01	-3.113	232.5	163.5	69.0	-29.677	215.0	144.5	70.5	-32.791
Healthy Climate(TM) UV-500	04/28/01	-6.526	219.2	182.3	36.9	-16.834	190.5	146.0	44.5	-23.360
BioFighter Triad 1S20	04/29/01	-13.413	200.4	192.3	8.1	-4.042	211.4	174.5	36.9	-17.455
BioFighterTriad 2S20	04/30/01	3.939	211.4	155.1	56.3	-26.632	212.4	164.2	48.2	-22.693

	Lights Off					Lights On					
			$^{\Delta}$ P Unit	$^{\Delta}$ P Nozzle	Flow Rate				$^{\Delta}$ P Unit	$^{\Delta}$ P Nozzle	Flow Rate
	Temp. °F	R.H.	(inches H2O)		(CFM)		Temp. °F	R.H.	(inche	s H2O)	(CFM)
Healthy Climate (TM) UV-2000	68.81	50.02	0.019	0.70	942.5		69.09	49.84	0.019	0.70	942.5
Healthy Climate (TM) UV-1000	77.02	44.98	0.021	0.67	923.9		78.83	49.84	0.020	0.67	923.9
Healthy Climate(TM) UV-500	71.28	47.06	0.018	0.69	936.4		70.96	47.79	0.018	0.69	936.4
BioFighter Triad 1S20	80.71	40.93	0.018	0.68	930.2		80.48	41.84	0.018	0.68	930.2
BioFighterTriad 2S20	73.41	58.91	0.018	0.68	930.2		73.46	59.32	0.019	0.69	936.4

Prepared by Henry Greist for Dust Free, Inc. 5/14/01



Table 2: Serratia marcescens UV Performance Data

Serratia marcescens

UV Light Comparison

				Light Off (cfu/cu.ft.)			Light On (cfu/cu.ft.)				
		% Kill from	% Kill after		After				After		
	Date	Upstream	Background		Diffusion		%		Diffusion		%
	Completed	Intensity	Subtracted	Before unit	Plate	Reduction	Reduction	Before unit	Plate	Reduction	Reduction
Healthy Climate (TM) UV-2000	05/03/01	-99.480	-100.000	269.0	138.8	130.2	-48.401	1.4	0.0	1.4	-100.000
Healthy Climate (TM) UV-1000	05/02/01	-96.893	-100.000	280.0	245.0	35.0	-12.500	8.7	0.0	8.7	-100.000
Healthy Climate(TM) UV-500	05/03/01	-98.425	-100.000	279.4	100.6	178.8	-63.994	4.4	0.0	4.4	-100.000
BioFighter Triad 1S20	05/02/01	-95.692	-100.000	220.5	152.2	68.3	-30.975	9.5	0.0	9.5	-100.000
BioFighterTriad 2S20	05/02/01	-99.049	-100.000	273.5	166.5	107.0	-39.122	2.6	0.0	2.6	-100.000

			[∆] P Unit	$^{\Delta}$ P Nozzle	Flow Rate
	Temp. °F	R.H.	(inches	H2O)	(CFM)
Healthy Climate (TM) UV-2000	72.06	69.23	0.021	0.70	942.5
Healthy Climate (TM) UV-1000	75.54	66.85	0.020	0.68	930.2
Healthy Climate(TM) UV-500	75.47	60.75	0.020	0.68	930.2
BioFighter Triad 1S20	77.21	63.05	0.020	0.68	930.2
BioFighterTriad 2S20	79.43	54.19	0.020	0.68	930.2

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Each organism has a specific protocol fur 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).