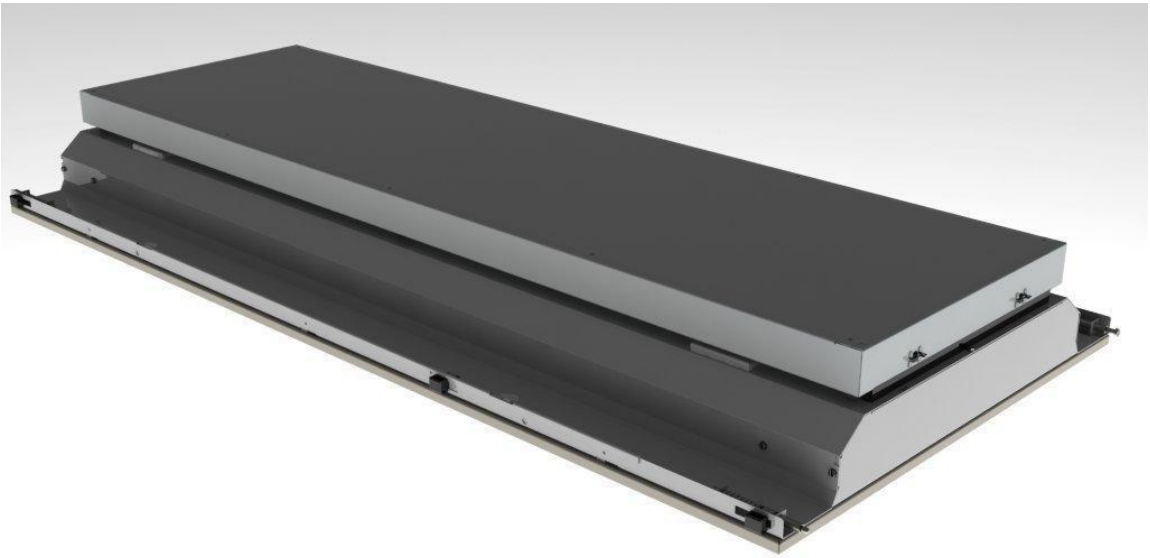
Report on the Performance of the

UV24 System



By Dr. Wladyslaw J. Kowalski

December 7, 2011

Medical Illumination - 547 Library Street, San Fernando, CA 91340

Nuvo - 5368 Kuhl Road, Pennsylvania,16510 (div of Medical Illumination)

Executive Summary

The superior performance of the UV24 System will enable high levels of air disinfection to be achieved as well as a consequent reduction in the risk of airborne nosocomial infection in any health care environment. This report evaluates the performance of the UV24 System in terms of the removal rates of known airborne nosocomial or hospital-acquired pathogens, including bacteria, viruses, and fungi. Removal rates due to filtration and UV irradiation are evaluated using computer models and tabulated for various operating conditions. These removal rates, which will reduce concentrations of airborne pathogens, are used to estimate the risk of infection to occupants and this risk is presented in terms of the Zonal Protection Factor (ZPF). The ZPF is a measure of the percentage of occupants protected form infection. Removal rates for most pathogens are in excess of 90% in the primary configurations. At the nominal design airflow of **50 cfm**, and with a **MERV 6** filter and a UV lamp with **15 W** of UV output, the UV24 System provides overall **single pass removal rates of approximately 97%** on the average for the 44 airborne nosocomial pathogens in the database. For the nominal design floor area of 100 ft^2, the unit will provide Zonal Protection Factors that exceed 91% for most of the pathogens in the database, with an **average ZPF of 90%**. ZPF data is provided for alternate airflows ranging from 50 ft^2 to 300 ft^2 as shown in Table 6. Both the single pass removal rates and the ZPFs are close to the limits of what can be achieved with a combined UV and filtration system, and this unit should outperform other similar units while consuming the least amount of energy as it has been optimized for both high performance and low power consumption. Quiet operation also makes this unit suitable for any hospital environment and it can be used in general wards, procedure rooms, hallways, laboratories, and other facilities.

INDEX

Introduction

The UV24 System is a UV air disinfection system incorporated into a ceiling light fixture such that it has no visible profile. It is intended to disinfect the air of bacteria, viruses, and fungi in hospital environments. The system includes an ultraviolet (UV) lamp, a filter, and a fan that will recirculate air locally, such as in a hallway or procedure room. Light baffles ensure that no hazardous levels of UV will escape into the local area. The system is designed to operate quietly while delivering air of the highest purity, with disinfection rates approaching 100%. Targeted microorganisms include MRSA, VRE, *Acinetobacter, Streptococcus* species, Influenza, VZV, *Pseudomonas, Clostridium difficile,* and other causative agents of hospital-acquired infections (HAIs). This report summarizes the predicted performance of the unit in terms of UV dose, filter removal rates, and overall disinfection or kill rates of pathogenic nosocomial microbes. Comparative predictions are provided for reductions of airborne concentrations of microbes for particular floor areas and room volumes. These airborne reductions are related to the risk of reduced infections. This unit will also remove fungal and bacterial spores at rates that are specified in this report.

System Description

The UV24 System consists of a ceiling light fixture that includes a UV lamp, a filter, light baffles, and a UV irradiation chamber. Air is drawn into the unit, filtered and disinfected, and then exhausted back into the local airspace. A MERV 6 filter is included to keep dust off the UV lamp, but this filter also serves to remove a percentage of airborne pathogens. Figure 1 shows a partially exploded view of the major components of the system. From top to bottom are shown the UV irradiation chamber, the lighting components, and the bottom grille.

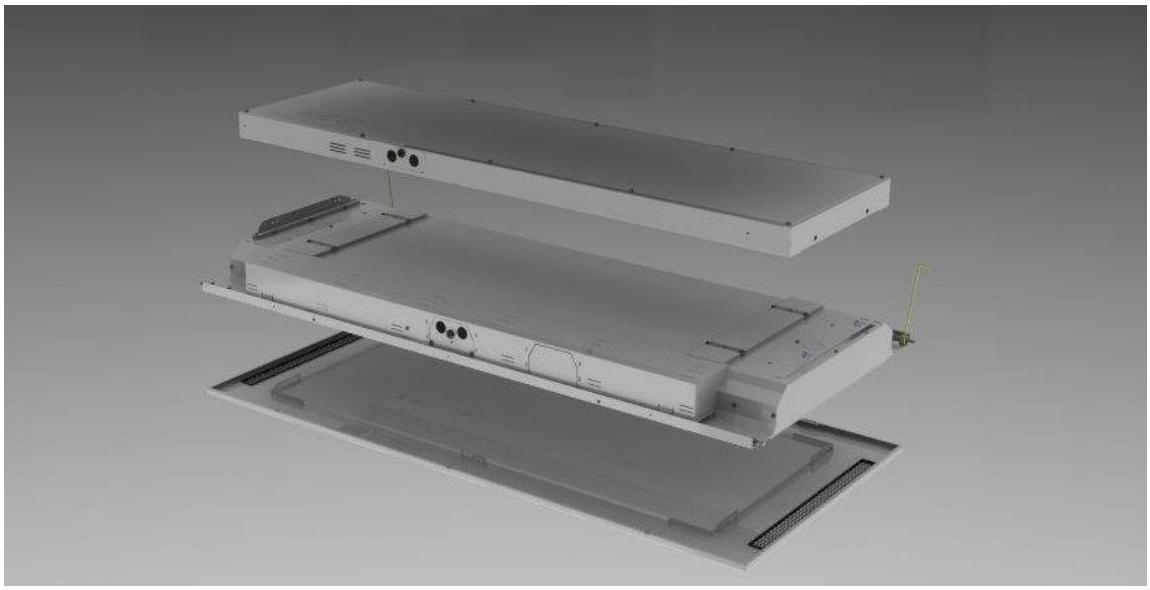


Figure 1: Partially exploded view of the UV24 System showing major components.

Figure 2 shows the internal arrangement of the UV irradiation chamber, including the UV lamps and the fans at the outlet. The irradiation chamber is lined or coated with reflective materials to increase the total irradiance.

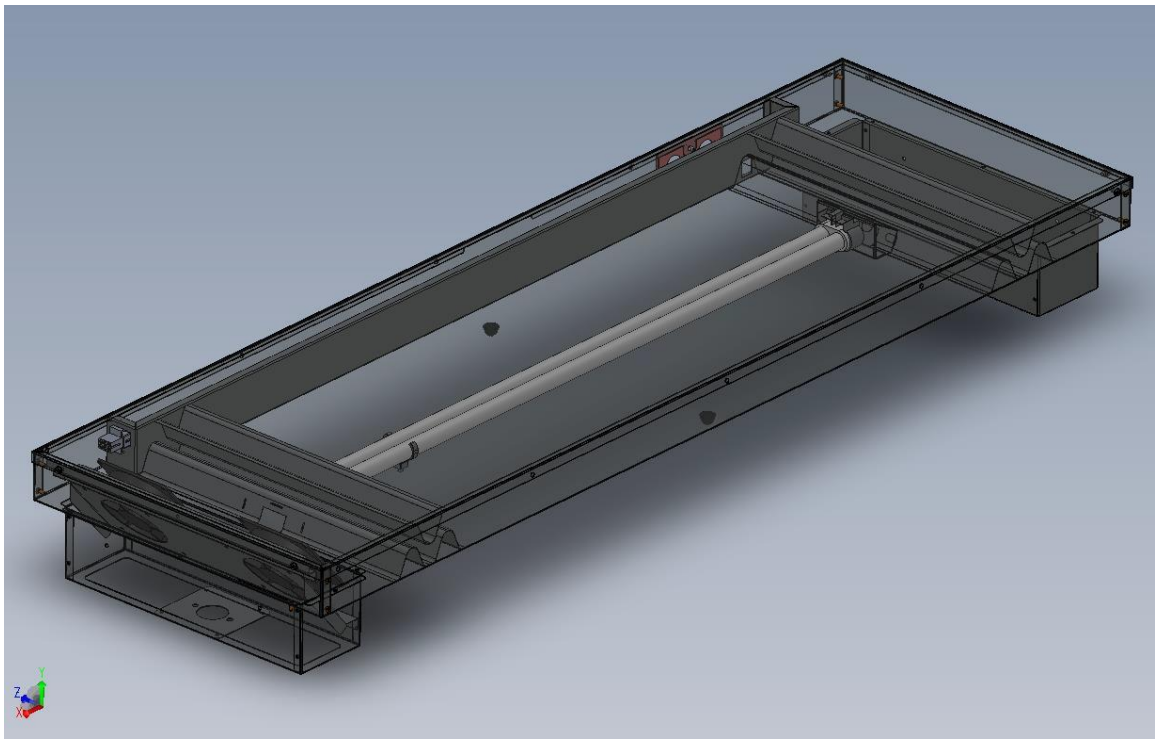


Figure 2: Schematic image of UV24 System showing internal UV lamps and irradiation chamber.

The properties of the reflective material were provided in the manufacturer's documents. The reflectivity of the aluminum surface is approximately 75-85% in the UV spectrum. Dimensions and design specifications are summarized in Table 1. These dimensions have been adjusted from those shown in the previous figure. The two configurations represent two different UV lamp sizes. The nominal airflow is 50 cfm. The airflow affects the air velocity and the exposure time (Et). Lamp dimensions and coordinates are unchanged from Table 1. At the nominal airflow of 50 scfm the air velocity is approximately 252 fpm. This is an acceptable airflow and will not decrease filter performance – in fact, filter performance will be slightly improved over the results given in the tables. The normal operating design airflow for a UV lamp is about 400-600 fpm.

Table 1: Specifications for UV24 System

Lamp Type	T5
# Lamps	1
Lamp UV Output, W	15
Width, in	14.5
Length, in	30.979
Height, in	1.97
Width, ft	1.21
Length, ft	2.58
Height, ft	0.16
Width, cm	36.83
Length, cm	78.69
Height, cm	5.0038
Nominal Airflow, Q, cfm	50
Nominal Airflow, Q, m^3/min	1.42
Velocity, fpm	252
Velocity, fps	4.20
Velocity, cm/s	128.04
Velocity, m/s	1.28
Face Area, in^2	28.57
Face Area, ft^2	0.1984
Face Area, cm^2	184.29
Face Area, m^2	0.0184
Exposure Time, Et, s	0.6145
lamp end coordinate, x1, cm	18.42
lamp end coordinate, x2, cm	18.42
lamp end coordinate, y1, cm	2.50
lamp end coordinate, y2, cm	2.50
lamp end coordinate, z1, cm	10.77
lamp end coordinate, z2, cm	67.92
lamp arc length, arcl, cm	57.15
lamp radius, r, cm	0.79

A MERV 6 filter is included in the unit and a representative filter performance curve is adapted from Kowalski and Bahnfleth (2002). Figure 3 shows the filter performance curve. The removal efficiency at any microbial particle size can be estimated from this curve.

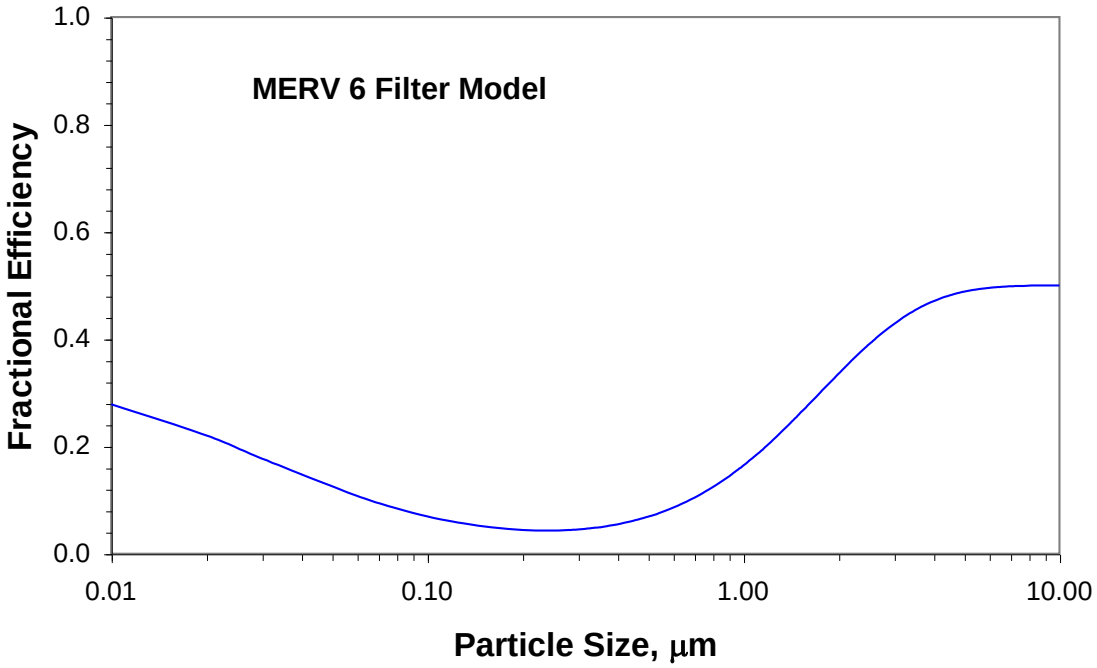


Figure 3: Performance of a generic MERV 6 filter. Based on modeling per Kowalski & Bahnfleth (2002).

Fan Pressure Loss and Noise

One of the design criteria for the UV24 System is that it must have quiet operation and produce the lowest possible level of noise. Noise is produced by the airflow as it moves through the fan and from turbulence inside the unit. The higher the pressure drop through the system the higher the fan static pressure and noise level. The unit includes smooth baffles to reduce turbulence inside the unit and fans have been selected for quiet operation. In order to minimize pressure losses a low pressure drop MERV 6 filter has been selected.

Analysis Results

Table 2 lists all nosocomial or HAI microbes that are known or suspected of having an airborne opponent in their transmission cycle. These include bacteria, viruses, fungal spores, and bacterial spores. Table 2 identifies the predicted filter removal rate for all these microbes based on the Figure 3 filter model. These filter removal rates will be combined with the UV disinfection rates to develop the overall removal rates of the pathogens. The filter removal rates are computed based on the log mean diameter of each pathogen. The size range of any microbe in nature will have a lognormal distribution, or a normal distribution (bell curve) on a logarithmic scale. The log mean diameter is an adequate representation of the mean size of any microbial population (Kowalski et al 1999). The log mean diameter will therefore be an adequate predictor of removal rates through any filter with a known performance curve.

Table 2: MERV 6 Filter Removal Rates of Airborne Nosocomial Pathogens

Microbe	Type	Size	MERV 6 Removal Rate	
		μm	fraction	%
Acinetobacter	Bacteria	1.225	0.2089	20.9
Adenovirus	Virus	0.079	0.0853	8.5
Aspergillus spores	Fungi	3.354	0.4476	44.8
Blastomyces dermatitidis spores	Fungi	12.649	0.5000	50.0
Bordetella pertussis	Bacteria	0.245	0.0431	4.3
Clostridium difficile spores	Bacteria	2	0.3353	33.5
Clostridium perfringens spores	Bacteria	1	0.1643	16.4
Coronavirus (SARS)	Virus	0.11	0.0643	6.4
Corynebacterium diphtheriae	Bacteria	0.698	0.1040	10.4
Coxsackievirus	Virus	0.027	0.1886	18.9
Cryptococcus neoformans spores	Fungi	4.899	0.4872	48.7
Enterobacter cloacae	Bacteria	1.414	0.2442	24.4
Enterococcus	Bacteria	1.414	0.2442	24.4
Fusarium spores	Fungi	11.225	0.5000	50.0
Haemophilus influenzae	Bacteria	0.285	0.0443	4.4
Haemophilus parainfluenzae	Bacteria	1.732	0.2973	29.7
Influenza A virus	Virus	0.098	0.0709	7.1
Klebsiella pneumoniae	Bacteria	0.671	0.0988	9.9
Legionella pneumophila	Bacteria	0.52	0.0721	7.2
Measles virus	Virus	0.158	0.0493	4.9
Mucor spores	Fungi	7.071	0.4983	49.8
Mumps virus	Virus	0.164	0.0483	4.8
Mycobacterium avium	Bacteria	1.118	0.1879	18.8
Mycobacterium tuberculosis	Bacteria	0.637	0.0925	9.3
Mycoplasma pneumoniae	Bacteria	0.177	0.0464	4.6
Neisseria meningitidis	Bacteria	0.775	0.1190	11.9
Nocardia asteroides	Bacteria	1.118	0.1879	18.8
Norwalk virus	Virus	0.029	0.1809	18.1
Parainfluenza virus	Virus	0.194	0.0447	4.5
Parvovirus B19	Virus	0.022	0.2104	21.0
Proteus mirabilis	Bacteria	0.494	0.0680	6.8
Pseudomonas aeruginosa	Bacteria	0.494	0.0680	6.8
Reovirus	Virus	0.075	0.0892	8.9
RSV	Virus	0.19	0.0450	4.5
Rhinovirus	Virus	0.023	0.2057	20.6
Rhizopus spores	Fungi	6.928	0.4981	49.8
Rotavirus	Virus	0.073	0.0913	9.1
Rubella virus	Virus	0.061	0.1062	10.6
Serratia marcescens	Bacteria	0.632	0.0916	9.2
Staphylococcus aureus	Bacteria	0.866	0.1372	13.7
Staphylococcus epidermis	Bacteria	0.866	0.1372	13.7
Streptococcus pneumoniae	Bacteria	0.707	0.1057	10.6
Streptococcus pyogenes	Bacteria	0.894	0.1428	14.3
VZV	Virus	0.173	0.0469	4.7

Appendix A reproduces the information in Table 2 but arranges the microbes in order of decreasing removal rates. Figure 4 illustrates how the pathogen removal rates correlate with the microbe size. This chart essentially duplicates Figure 4, the performance curve for a MERV 6 filter.

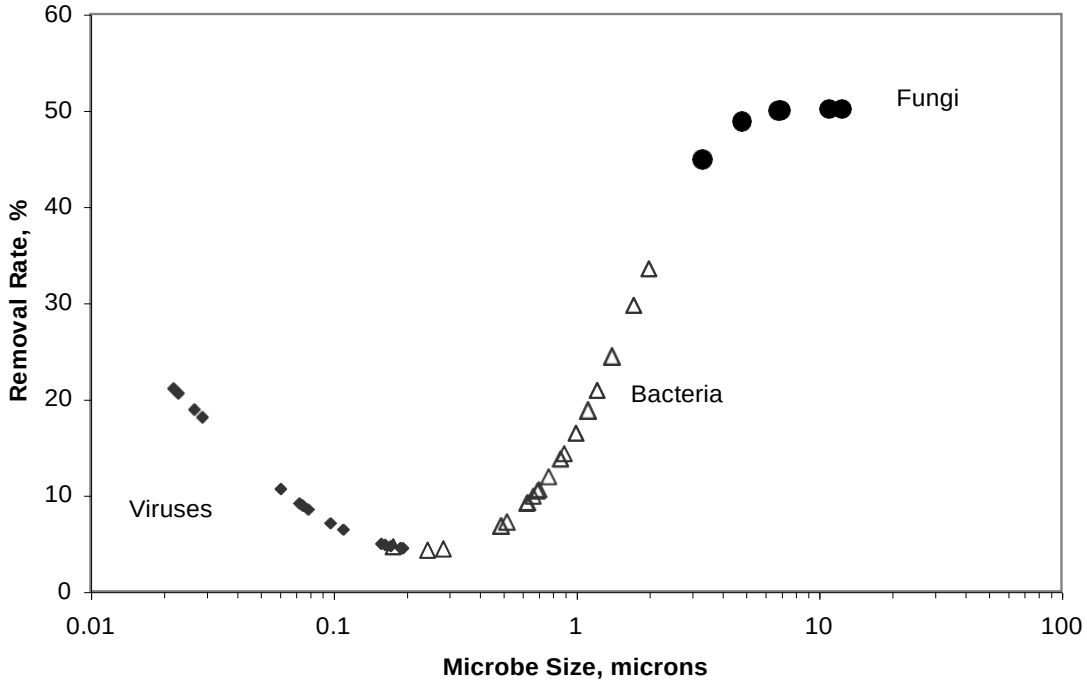


Figure 4: Removal rates for nosocomial pathogens arranged in a MERV 6 filter performance curve format (see Figure 3).

Table 3 lists the disinfection rates for airborne nosocomial pathogens based on the dose produced by the UV lamp. The 15 W lamp has been modeled (using the methods from Kowalski 2009) and found to produce a UV dose of 198 J/m^2 at 50 cfm. This dose is used to compute the disinfection rate based on the indicated UV rate constants listed in Table 3. Table 3 is reproduced in Appendix B where the pathogens are listed in order of decreasing removal rates.

Table 3: UV Disinfection Rates of Airborne Nosocomial Pathogens

Microbe	Type	UV k m^2/J	UV D90 J/m^2	Disinfection Rate, % 15 W
Acinetobacter	Bacteria	0.16	14	100
Adenovirus	Virus	0.054	43	100
Aspergillus spores	Fungi	0.00894	258	83
Blastomyces dermatitidis spores	Fungi	0.01645	140	96
Bordetella pertussis*	Bacteria	0.0364	63	100
Clostridium difficile spores	Bacteria	0.0385	60	100
Clostridium perfringens spores	Bacteria	0.0385	60	100
Coronavirus (SARS)	Virus	0.377	6	100
Corynebacterium diphtheriae	Bacteria	0.0701	33	100
Coxsackievirus	Virus	0.111	21	100
Cryptococcus neoformans spores	Fungi	0.0167	138	96
Enterobacter cloacae	Bacteria	0.03598	64	100
Enterococcus*	Bacteria	0.0822	28	100
Fusarium spores	Fungi	0.00855	269	82
Haemophilus influenzae	Bacteria	0.11845	19	100
Haemophilus parainfluenzae*	Bacteria	0.03	77	100
Influenza A virus	Virus	0.119	19	100
Klebsiella pneumoniae	Bacteria	0.04435	52	100
Legionella pneumophila	Bacteria	0.2024	11	100
Measles virus	Virus	0.1051	22	100
Mucor spores	Fungi	0.01012	228	87
Mumps virus*	Virus	0.0766	30	100
Mycobacterium avium	Bacteria	0.04387	52	100
Mycobacterium tuberculosis	Bacteria	0.4721	5	100
Mycoplasma pneumoniae	Bacteria	0.2791	8	100
Neisseria meningitidis*	Bacteria	0.1057	22	100
Nocardia asteroides	Bacteria	0.0822	28	100
Norwalk virus*	Virus	0.0116	198	90
Parainfluenza virus*	Virus	0.1086	21	100
Parvovirus B19	Virus	0.092	25	100
Proteus mirabilis	Bacteria	0.289	8	100
Pseudomonas aeruginosa	Bacteria	0.5721	4	100
Reovirus	Virus	0.01459	158	94
RSV*	Virus	0.0917	25	100
Rhinovirus*	Virus	0.0142	162	94
Rhizopus spores	Fungi	0.00861	267	82
Rotavirus	Virus	0.02342	98	99
Rubella virus*	Virus	0.0037	622	52
Serratia marcescens	Bacteria	0.221	10	100
Staphylococcus aureus	Bacteria	0.5957	4	100
Staphylococcus epidermis	Bacteria	0.09703	24	100
Streptococcus pneumoniae	Bacteria	0.00492	468	62
Streptococcus pyogenes	Bacteria	0.8113	3	100
VZV (Varicella surrogate k)	Virus	0.1305	18	100
UV Dose, J/m^2		198		

Note: Asterisk means the UV rate constant is a predicted value based on the complete genome.

Table 4 lists the combined single pass removal rates for the array of nosocomial pathogens in the previous tables. This table is shown again in Appendix C where they have been rearranged in order of most susceptible to least susceptible.

Given the removal rates as listed in Table 4, the Zonal Protection Factor (ZPF) can be computed assuming the floor area coverage is 100 ft^2 and the zone has 15% outside air (from the normal ventilation system). The ZPF (aka BPF or Building Protection Factor) represents the theoretical protection offered to occupants by the UV24 System. It must be computed for each individual pathogen since each pathogen has a different removal rate (RR). The ZPF is computed by transient analysis of the airborne concentrations in the zone and is based on eight hours of breathing air in the occupied zone. The ZPF is the percentage of occupants likely to be protected from infection. The converse of the ZPF (1-ZPF) represents the number of likely infections. It is computed by assuming that the baseline condition has no air cleaning. The baseline condition assumes a release rate of pathogens sufficient to cause 99% infections. See Kowalski (2009) or Kowalski (2006) for more specific details on the computation of the BPF or ZPF.

Table 5 summarizes the predicted ZPF for each of the nosocomial pathogens under consideration and it indicates that very high protection factors are possible with the UV24 System, which could be expected since the removal rates are high for most pathogens and the airflow rate (50 cfm) is appropriate for the subject floor area (100 ft^2). The maximum ZPF is 93% and this cannot be increased further without increasing airflow. The minimum acceptable ZPF for any system is about 50%, which means that half the occupants are protected from infection. Based on the results in Table 5, the ZPF is above the minimum for all pathogens, including bacterial and fungal spores.

The results in Table 5 are based on the nominal airflow rate of 50 cfm. The results will be proportionally lower for lower airflow rates, since the combined removal rates (filters plus UV) are largely maxed out. Table 5 is reproduced in Appendix C but rearranged in order of decreasing ZPF.

Table 4: Combined Single Pass Removal Rates

Microbe	Component Removal Rates		Overall Removal Rates
	MERV 6	15 W	MERV 6 + 15 W
	fraction	fraction	%
Acinetobacter	0.2089	1.0000	100.0
Adenovirus	0.0853	1.0000	100.0
Aspergillus spores	0.4476	0.8297	90.6
Blastomyces dermatitidis spores	0.5000	0.9615	98.1
Bordetella pertussis	0.0431	0.9993	99.9
Clostridium difficile spores	0.3353	0.9995	100.0
Clostridium perfringens spores	0.1643	0.9995	100.0
Coronavirus (SARS)	0.0643	1.0000	100.0
Corynebacterium diphtheriae	0.1040	1.0000	100.0
Coxsackievirus	0.1886	1.0000	100.0
Cryptococcus neoformans spores	0.4872	0.9634	98.1
Enterobacter cloacae	0.2442	0.9992	99.9
Enterococcus	0.2442	1.0000	100.0
Fusarium spores	0.5000	0.8160	90.8
Haemophilus influenzae	0.0443	1.0000	100.0
Haemophilus parainfluenzae	0.2973	0.9974	99.8
Influenza A virus	0.0709	1.0000	100.0
Klebsiella pneumoniae	0.0988	0.9998	100.0
Legionella pneumophila	0.0721	1.0000	100.0
Measles virus	0.0493	1.0000	100.0
Mucor spores	0.4983	0.8652	93.2
Mumps virus	0.0483	1.0000	100.0
Mycobacterium avium	0.1879	0.9998	100.0
Mycobacterium tuberculosis	0.0925	1.0000	100.0
Mycoplasma pneumoniae	0.0464	1.0000	100.0
Neisseria meningitidis	0.1190	1.0000	100.0
Nocardia asteroides	0.1879	1.0000	100.0
Norwalk virus	0.1809	0.8994	91.8
Parainfluenza virus	0.0447	1.0000	100.0
Parvovirus B19	0.2104	1.0000	100.0
Proteus mirabilis	0.0680	1.0000	100.0
Pseudomonas aeruginosa	0.0680	1.0000	100.0
Reovirus	0.0892	0.9444	94.9
RSV	0.0450	1.0000	100.0
Rhinovirus	0.2057	0.9399	95.2
Rhizopus spores	0.4981	0.8182	90.9
Rotavirus	0.0913	0.9903	99.1
Rubella virus	0.1062	0.5193	57.0
Serratia marcescens	0.0916	1.0000	100.0
Staphylococcus aureus	0.1372	1.0000	100.0
Staphylococcus epidermis	0.1372	1.0000	100.0
Streptococcus pneumoniae	0.1057	0.6225	66.2
Streptococcus pyogenes	0.1428	1.0000	100.0
VZV	0.0469	1.0000	100.0

Table 5: Zonal Protection Factors for Nosocomial Pathogens

Pathogen	Type	MERV6+15W	FA 100 ft^2
		Removal %	ZPF %
Acinetobacter	Bacteria	100.0	91
Adenovirus	Virus	100.0	91
Aspergillus spores	Fungi	90.6	89
Blastomyces dermatitidis spores	Fungi	98.1	90
Bordetella pertussis	Bacteria	99.9	91
Clostridium difficile spores	Bacteria	100.0	91
Clostridium perfringens spores	Bacteria	100.0	91
Coronavirus (SARS)	Virus	100.0	91
Corynebacterium diphtheriae	Bacteria	100.0	91
Coxsackievirus	Virus	100.0	91
Cryptococcus neoformans spores	Fungi	98.1	90
Enterobacter cloacae	Bacteria	99.9	91
Enterococcus	Bacteria	100.0	91
Fusarium spores	Fungi	90.8	89
Haemophilus influenzae	Bacteria	100.0	91
Haemophilus parainfluenzae	Bacteria	99.8	91
Influenza A virus	Virus	100.0	91
Klebsiella pneumoniae	Bacteria	100.0	91
Legionella pneumophila	Bacteria	100.0	91
Measles virus	Virus	100.0	91
Mucor spores	Fungi	93.2	89
Mumps virus	Virus	100.0	91
Mycobacterium avium	Bacteria	100.0	91
Mycobacterium tuberculosis	Bacteria	100.0	91
Mycoplasma pneumoniae	Bacteria	100.0	91
Neisseria meningitidis	Bacteria	100.0	91
Nocardia asteroides	Bacteria	100.0	91
Norwalk virus	Virus	91.8	89
Parainfluenza virus	Virus	100.0	91
Parvovirus B19	Virus	100.0	91
Proteus mirabilis	Bacteria	100.0	91
Pseudomonas aeruginosa	Bacteria	100.0	91
Reovirus	Virus	94.9	90
Rhinovirus	Virus	100.0	91
Rhizopus spores	Virus	95.2	90
Rotavirus	Fungi	90.9	89
RSV	Virus	99.1	91
Rubella virus	Virus	57.0	73
Serratia marcescens	Bacteria	100.0	91
Staphylococcus aureus	Bacteria	100.0	91
Staphylococcus epidermis	Bacteria	100.0	91
Streptococcus pneumoniae	Bacteria	66.2	79
Streptococcus pyogenes	Bacteria	100.0	91
VZV	Virus	100.0	91
Average Protection Factor %			90

Table 6 provides the Zonal Protection Factors for a range of alternate airflows from 50 ft^2 to 300 ft^2, arranged in order of descending ZPF.

Table 6: Zonal Protection Factors for Nosocomial Pathogens at Alternate Room Areas

Pathogen	MERV6+15W	Zonal Protection Factor %						
	Removal	Area	Area	Area	Area	Area	Area	Area
	%	50 ft^2	75 ft^2	100 ft^2	125 ft^2	150 ft^2	200 ft^2	300 ft^2
Coronavirus (SARS)	100.0	93	92	91	90	88	85	80
Legionella pneumophila	100.0	93	92	91	90	88	85	80
Mycobacterium tuberculosis	100.0	93	92	91	90	88	85	80
Mycoplasma pneumoniae	100.0	93	92	91	90	88	85	80
Proteus mirabilis	100.0	93	92	91	90	88	85	80
Pseudomonas aeruginosa	100.0	93	92	91	90	88	85	80
Serratia marcescens	100.0	93	92	91	90	88	85	80
Staphylococcus aureus	100.0	93	92	91	90	88	85	80
Streptococcus pyogenes	100.0	93	92	91	90	88	85	80
Acinetobacter	100.0	93	92	91	90	88	85	80
VZV	100.0	93	92	91	90	88	85	80
Influenza A virus	100.0	93	92	91	90	88	85	80
Haemophilus influenzae	100.0	93	92	91	90	88	85	80
Coxsackievirus	100.0	93	92	91	90	88	85	80
Parainfluenza virus	100.0	93	92	91	90	88	85	80
Neisseria meningitidis	100.0	93	92	91	90	88	85	80
Measles virus	100.0	93	92	91	90	88	85	80
Staphylococcus epidermis	100.0	93	92	91	90	88	85	80
Parvovirus B19	100.0	93	92	91	90	88	85	80
Rhinovirus	100.0	93	92	91	90	88	85	80
Enterococcus	100.0	93	92	91	90	88	85	80
Nocardia asteroides	100.0	93	92	91	90	88	85	80
Mumps virus	100.0	93	92	91	90	88	85	80
Corynebacterium diphtheriae	100.0	93	92	91	90	88	85	80
Adenovirus	100.0	93	92	91	90	88	85	80
Mycobacterium avium	100.0	93	92	91	90	88	85	80
Klebsiella pneumoniae	100.0	93	92	91	90	88	85	80
Clostridium difficile spores	100.0	93	92	91	90	88	85	80
Clostridium perfringens spores	100.0	93	92	91	90	88	85	80
Enterobacter cloacae	99.9	93	92	91	90	88	85	80
Bordetella pertussis	99.9	93	92	91	90	88	85	80
Haemophilus parainfluenzae	99.8	93	92	91	90	88	85	79
RSV	99.1	93	92	91	89	88	85	79
Cryptococcus neoformans spores	98.1	93	92	90	89	88	85	79
Blastomyces dermatitidis spores	98.1	93	92	90	89	88	85	79
Rhizopus spores	95.2	92	91	90	88	87	84	78
Reovirus	94.9	92	91	90	88	87	84	78
Mucor spores	93.2	92	91	89	88	86	83	77
Norwalk virus	91.8	92	90	89	87	86	83	76
Rotavirus	90.9	91	90	89	87	86	82	76
Fusarium spores	90.8	91	90	89	87	86	82	76
Aspergillus spores	90.6	91	90	89	87	86	82	76
Streptococcus pneumoniae	66.2	83	81	79	77	74	69	61
Rubella virus	57.0	78	75	73	70	67	62	53

Summary and Discussion of Analysis Results

The analysis presented herein indicates that the UV24 System will produce high removal rates of all nosocomial pathogens including bacteria, fungi, and viruses. Analysis also shows that the risk of infection in areas where the unit is installed will be considerably reduced, based on predicted reduction in airborne concentrations of microbes. The combination of a 15 W UV lamp, a MERV 6 filter, and an airflow rate of 50 cfm will produce an average removal rates in excess of 97%, in a single pass, for all but two of the pathogens listed in Table 5. Table 7 provides a comparison of unitary UV systems operating between 30-100 cfm arranged in order of UV dose. The UV24 System provides a higher UV dose than most of these systems and has more efficient filtration than all but one of these systems.

Table 7:Comparison of Unitary UV Systems between 30-100 cfm

Manufacturer	Model	Airflow/CADR		Prefilter	Primary Filter	UVP W	Dose J/m^2	URV	Notes
		cfm	m^3/min						
Virobuster	Steritube	44	1.25	G4	none	57	592	23	multispeed
Virobuster	Steritube	59	1.67	G4	none	57	443	22	multispeed
Virobuster	Steritube	74	2.083	G4	none	57	355	21	multispeed
sterilAir AG	LSK2036-U	29.2	0.83	none	none	30	315	21	
MI/Nuvo	**UV24 System**	**50**	**1.42**	**Yes**	**MERV6**	**15**	**198**	**19**	
sterilAir AG	UVR2250-1	88	2.50	optional	none	27	190	19	
sterilAir AG	LSK2018	11.8	0.33	none	none	7	173	19	
Holmes Group	BAP920-U	100	2.83	none	MERV15	22	69.2	17	PCO
Sanuvox	P-900	35	0.99	Yes	none	4.76	48	15	multispeed
Amcor	AM-45	30	0.85	none	none	1.96	26.9	13	
Amcor	AM-45C	31	0.88	Yes	none	25	23.2	13	PCO, carbon
Amcor	AM-45C	40	1.13	Yes	none	25	17.8	12	PCO, carbon
Amcor	AM-45C	45	1.27	Yes	none	25	15.9	12	PCO, carbon
Amcor	AM-45	60	1.70	none	none	1.96	13.5	11	

NOTE: CADR is approximately equal to the airflow for all systems.

References & Bibliography

Kowalski, W. J., W. P. Bahnfleth, T. S. Whittam (1999). "Filtration of Airborne Microorganisms: Modeling and prediction." *ASHRAE Transactions* 105(2), 4-17. http://www.engr.psu.edu/ae/wjk/fom.html.

Kowalski, W. J., and Bahnfleth, W. P. (2000). "UVGI Design Basics for Air and Surface Disinfection." *HPAC* 72(1), 100-110.

Kowalski, W. J., and Bahnfleth, W. P. (2002). "MERV filter models for aerobiological applications." *Air Media* Summer, 13-17.

Kowalski, W. J., and Bahnfleth, W. P. (2004). "Proposed Standards and Guidelines for UVGI Air Disinfection." *IUVA News* 6(1), 20-25.

Kowalski, W. J., Bahnfleth, W. P., and Mistrick, R. G. (2005). "A specular model for UVGI air disinfection systems." *IUVA News* 7(1), 19-26.

Kowalski, W. J. (2006). *Aerobiological Engineering Handbook: A Guide to Airborne Disease Control Technologies.* McGraw-Hill, New York.

Kowalski, W. J. (2007). "Air-Treatment Systems for Controlling Hospital-Acquired Infections." *HPAC Engineering* 79(1), 28-48.

Kowalski, W. J. (2009). *Ultraviolet Germicidal Irradiation Handbook: UVGI for Air and Surface Disinfection.* Springer, New York.

Kowalski, W. J. (2011). *Hospital Airborne Infection Control.* Taylor & Francis/CRC Press, New York.

Appendix A: Filter Removal Rates of Airborne Nosocomial Pathogens

Microbe	Type	Size μm	Removal Rate % MERV 6
Blastomyces dermatitidis spores	Fungi	12.649	50.0
Fusarium spores	Fungi	11.225	50.0
Mucor spores	Fungi	7.071	49.8
Rhizopus spores	Fungi	6.928	49.8
Cryptococcus neoformans spores	Fungi	4.899	48.7
Aspergillus spores	Fungi	3.354	44.8
Clostridium difficile spores	Bacteria	2	33.5
Haemophilus parainfluenzae	Bacteria	1.732	29.7
Enterobacter cloacae	Bacteria	1.414	24.4
Enterococcus	Bacteria	1.414	24.4
Acinetobacter	Bacteria	1.225	20.9
Mycobacterium avium	Bacteria	1.118	18.8
Nocardia asteroides	Bacteria	1.118	18.8
Clostridium perfringens spores	Bacteria	1	16.4
Streptococcus pyogenes	Bacteria	0.894	14.3
Staphylococcus aureus	Bacteria	0.866	13.7
Staphylococcus epidermis	Bacteria	0.866	13.7
Neisseria meningitidis	Bacteria	0.775	11.9
Streptococcus pneumoniae	Bacteria	0.707	10.6
Corynebacterium diphtheriae	Bacteria	0.698	10.4
Klebsiella pneumoniae	Bacteria	0.671	9.9
Mycobacterium tuberculosis	Bacteria	0.637	9.3
Serratia marcescens	Bacteria	0.632	9.2
Legionella pneumophila	Bacteria	0.52	7.2
Proteus mirabilis	Bacteria	0.494	6.8
Pseudomonas aeruginosa	Bacteria	0.494	6.8
Haemophilus influenzae	Bacteria	0.285	4.4
Bordetella pertussis	Bacteria	0.245	4.3
Parainfluenza virus	Virus	0.194	4.5
RSV	Virus	0.19	4.5
Mycoplasma pneumoniae	Bacteria	0.177	4.6
VZV	Virus	0.173	4.7
Mumps virus	Virus	0.164	4.8
Measles virus	Virus	0.158	4.9
Coronavirus (SARS)	Virus	0.11	6.4
Influenza A virus	Virus	0.098	7.1
Adenovirus	Virus	0.079	8.5
Reovirus	Virus	0.075	8.9
Rotavirus	Virus	0.073	9.1
Rubella virus	Virus	0.061	10.6
Norwalk virus	Virus	0.029	18.1
Coxsackievirus	Virus	0.027	18.9
Rhinovirus	Virus	0.023	20.6
Parvovirus B19	Virus	0.022	21.0

Appendix B: UV Disinfection Rates of Airborne Nosocomial Pathogens

Microbe	Type	UV k m^2/J	UV D90 J/m^2	Disinfection Rate, % 15 W
Coronavirus (SARS)	Virus	0.377	6	100
Legionella pneumophila	Bacteria	0.2024	11	100
Mycobacterium tuberculosis	Bacteria	0.4721	5	100
Mycoplasma pneumoniae	Bacteria	0.2791	8	100
Proteus mirabilis	Bacteria	0.289	8	100
Pseudomonas aeruginosa	Bacteria	0.5721	4	100
Serratia marcescens	Bacteria	0.221	10	100
Staphylococcus aureus	Bacteria	0.5957	4	100
Streptococcus pyogenes	Bacteria	0.8113	3	100
Acinetobacter	Bacteria	0.16	14	100
VZV (Varicella surrogate k)	Virus	0.1305	18	100
Influenza A virus	Virus	0.119	19	100
Haemophilus influenzae	Bacteria	0.11845	19	100
Coxsackievirus	Virus	0.111	21	100
Parainfluenza virus*	Virus	0.1086	21	100
Neisseria meningitidis*	Bacteria	0.1057	22	100
Measles virus	Virus	0.1051	22	100
Staphylococcus epidermis	Bacteria	0.09703	24	100
Parvovirus B19	Virus	0.092	25	100
RSV*	Virus	0.0917	25	100
Enterococcus*	Bacteria	0.0822	28	100
Nocardia asteroides	Bacteria	0.0822	28	100
Mumps virus*	Virus	0.0766	30	100
Corynebacterium diphtheriae	Bacteria	0.0701	33	100
Adenovirus	Virus	0.054	43	100
Klebsiella pneumoniae	Bacteria	0.04435	52	100
Mycobacterium avium	Bacteria	0.04387	52	100
Clostridium difficile spores	Bacteria	0.0385	60	100
Clostridium perfringens spores	Bacteria	0.0385	60	100
Bordetella pertussis*	Bacteria	0.0364	63	100
Enterobacter cloacae	Bacteria	0.03598	64	100
Haemophilus parainfluenzae*	Bacteria	0.03	77	100
Rotavirus	Virus	0.02342	98	99
Cryptococcus neoformans spores	Fungi	0.0167	138	96
Blastomyces dermatitidis spores	Fungi	0.01645	140	96
Reovirus	Virus	0.01459	158	94
Rhinovirus*	Virus	0.0142	162	94
Norwalk virus*	Virus	0.0116	198	90
Mucor spores	Fungi	0.01012	228	87
Aspergillus spores	Fungi	0.00894	258	83
Rhizopus spores	Fungi	0.00861	267	82
Fusarium spores	Fungi	0.00855	269	82
Streptococcus pneumoniae	Bacteria	0.00492	468	62
Rubella virus*	Virus	0.0037	622	52
UV Dose, J/m^2		198		

Note: Asterisk means the UV rate constant is a predicted value based on the complete genome.

Appendix C: Combined Single Pass Removal Rates

Microbe	Component Removal Rates		Overall Removal Rates
	MERV 6	15 W	MERV 6 + 15 W
	fraction	fraction	%
Coronavirus (SARS)	0.0643	1.0000	100.0
Legionella pneumophila	0.0721	1.0000	100.0
Mycobacterium tuberculosis	0.0925	1.0000	100.0
Mycoplasma pneumoniae	0.0464	1.0000	100.0
Proteus mirabilis	0.0680	1.0000	100.0
Pseudomonas aeruginosa	0.0680	1.0000	100.0
Serratia marcescens	0.0916	1.0000	100.0
Staphylococcus aureus	0.1372	1.0000	100.0
Streptococcus pyogenes	0.1428	1.0000	100.0
Acinetobacter	0.2089	1.0000	100.0
VZV	0.0469	1.0000	100.0
Influenza A virus	0.0709	1.0000	100.0
Haemophilus influenzae	0.0443	1.0000	100.0
Coxsackievirus	0.1886	1.0000	100.0
Parainfluenza virus	0.0447	1.0000	100.0
Neisseria meningitidis	0.1190	1.0000	100.0
Measles virus	0.0493	1.0000	100.0
Staphylococcus epidermis	0.1372	1.0000	100.0
Parvovirus B19	0.2104	1.0000	100.0
RSV	0.0450	1.0000	100.0
Enterococcus	0.2442	1.0000	100.0
Nocardia asteroides	0.1879	1.0000	100.0
Mumps virus	0.0483	1.0000	100.0
Corynebacterium diphtheriae	0.1040	1.0000	100.0
Adenovirus	0.0853	1.0000	100.0
Mycobacterium avium	0.1879	0.9998	100.0
Klebsiella pneumoniae	0.0988	0.9998	100.0
Clostridium difficile spores	0.3353	0.9995	100.0
Clostridium perfringens spores	0.1643	0.9995	100.0
Enterobacter cloacae	0.2442	0.9992	99.9
Bordetella pertussis	0.0431	0.9993	99.9
Haemophilus parainfluenzae	0.2973	0.9974	99.8
Rotavirus	0.0913	0.9903	99.1
Cryptococcus neoformans spores	0.4872	0.9634	98.1
Blastomyces dermatitidis spores	0.5000	0.9615	98.1
Rhinovirus	0.2057	0.9399	95.2
Reovirus	0.0892	0.9444	94.9
Mucor spores	0.4983	0.8652	93.2
Norwalk virus	0.1809	0.8994	91.8
Rhizopus spores	0.4981	0.8182	90.9
Fusarium spores	0.5000	0.8160	90.8
Aspergillus spores	0.4476	0.8297	90.6
Streptococcus pneumoniae	0.1057	0.6225	66.2
Rubella virus	0.1062	0.5193	57.0

BIOLOGICAL INACTIVATION OF UV24 SYSTEM

FINAL REPORT

PROTOCOL NO. AER 09112013 Rev 3

LABORATORY PROJECT ID #13016311

Organism: *Bacillus atrophaeus*

Contact time: 1 hr., 4hr, 8 hr. and 24 hr.

SUBMITTED BY

AEROBIOLOGY LABORATORY
43760 Trade Center Place, Suite 100
Dulles, VA 20166
Tel (703) 648-9150
Fax (703) 648-0963
e-mail: lab@aerobiology.net

BIOLOGICAL INACTIVATION OF THE UV24 SYSTEM

COMPLIANCE STATEMENT

Information on the structure and activation of UV24 system resides with the sponsor of the study.

QAU STATEMENT

TITLE: Biological Inactivation of UV24 (UV24)

PROTOCOL	AER 09112013 Rev 3
LAB PROJECT	**13016311**
EVALUATION STUDY	Study II
TEST DATE	10.27.2013
DATE QAU INSPECTED	10.28.2013
DATE REPORTED	10.29.2013

STUDY OBJECTIVES

Evaluate the inactivation efficiency of the UV24 system in terms of % reduction or Log reduction of microorganism in a simulated room in the same manner as the critical care facility areas in hospital settings. The system is designed to operate quietly with 100% disinfection while delivering highly purified air. The protocol described herein determines the inactivation efficiency of the system against spore forming and vegetative bacteria which represents a wide range of applications.

The test was conducted based on the EPA protocol "Biological Inactivation Efficiency by HVAC In-Duct Ultraviolet Light Systems". The Quality Assurance Unit of Aerobiology Laboratory has inspected the lab project # 13016311 in compliance with the current GLP Regulations and the reported results accurately reflect the raw data.

INTRODUCTION

This report describes the procedure and results of the biological inactivation efficiency of the UV24 system during an increased challenge of aerosolized inoculum of clinically relevant microorganisms. A medical grade nebulizer is used to deliver the accurate and consistent number of pathogens into the simulated room. The UV24 system is turned on for specified contact time during which the aerosolized microorganism comes in contact with the UV system resulting in reduction and release of purified air into the room. This test procedure is developed for a challenge level of greater than 10"8 colony forming units (CFU), which provides 1000 times higher challenge than would be expected in normal use. This method employed the fundamentals of the EPA method "Biological Inactivation Efficiency by HVAC In-Duct Ultraviolet Light Systems".

JUSTIFICATION

The surface air sampler (SAS) or impactor provides a number of advantages over other methods for test because it can simulate inhalation of aerosolized contaminants at controlled air flow rate. The SAS pulls in 500 I of air which is represented as 219 impaction holes on the media placed within the SAS. High volume of air causes multiple impaction which skews the data interpretation. The use of medical grade delivery nebulizer allows a high concentration of aerosol challenge to be aerosolized into the room. The actual count of challenging microorganism is predetermined by colony count, while the volume of the challenge aerosol is tightly controlled by monitoring the air flow and air pressure through the nebulizer. Aerosols are self-contained and they do not pose any bio-safety concerns.

DESCRIPTION OF TEST PROCEDURE

Sample conditioning and calibration

The Zipwall® is constructed as per manufacture instruction. The spacing of the poles is based on the movement of air in the room. The air flow is typical for commercial air space environment. Four glove box areas are specified in the Zipwall® room for nebulizer placement and for 3 SAS samplers. The UV24 unit is installed in the ceiling as per instructions and plugged in. At nominal design airflow of 50 cfm, with a MERV 6 filter and a UV lamp, the UV24 emits 15 watts of UV radiation at 253.7 nm. The SAS and the nebulizer are placed at the appropriate glove box. The SAS will operate at the flow rate of 100 1pm for 5 minutes for a total of 500 L.

Challenge phase

Unplug the UV24 system. Connect nebulizer to the PARI compressor. An appropriate media plate is also placed in each of the SAS. Add 8ml of inoculum to the sterile nebulizer. Attach the nebulizer to the connector and to the hose of the PARI compressor. Set timer for 15 minute. Turn on PARI compressor. The nebulizer aerosolizes the inoculum and the compressor is turned off after 15 min. The SAS are turned on for 5 min for sample collection. Three sets of samples are taken outside the simulated clean room using the SAS at 5 min to verify contamination of outside air by the indicator organism.

Contact time

The UV24 system is turned on after the baseline samples are taken. Samples are taken after the contact times of 1hr, 4 hr, 8 hr and 24 hr during, which the aerosolized inoculum comes in contact with UV24 system.

Recovery phase

After sampling at specified time intervals, the plates are then placed in the incubator at 35°C for 24 hrs to recover the challenge microorganism.

Plate count/ Result recording

Examine plates after 24 hours for growth, and colony count. If necessary, incubate plates for an additional 24 hours. After the colonies have grown, choose countable plate. Record control counts to determine the accuracy of the challenge and the inhibitory efficiency of the UV24 system. the raw colony counts are compared to the 219-hole surface air sampler (SAS) positive hole correction chart to get positive hole corrected colony count for the respective samples (Attachment 1).

TEST MATERIALS

Testing Area (Room area)	8ft x9ft x10ft
Equipment Source	UV24 System
Equipment	Surface air sampler
Date Received	(SAS) 10.21.13
Start Date	10.27.13
Lab study completion date	10.29.13
Report Issue Date	10.29.13

TESTS CONDITIONS

Test Matrix	6 samples
Organism	*Bacillus atrophaeus* ATCC 9372
Inoculum	10^8 cfu/ml
Media	Tryptic Soy Agar with 5% sheep's blood
Set up	Protocol no. 09112013 Rev 3 (Diagram 1)
Challenge Device	Nebulizer Pro NEB ULTRA II
Delivery Time	15 min
Challenge Volume	8ml
Internal Control	Pre-samples taken outside and inside the Zipwall room
"Base line"	Immediately after aerosolization for 15 min with UV24 off
Contact time	1hr, 4hr, 8hr, and 24hr
Incubator	35˚c
Recovery	24hr in Incubator at 35˚C

CALCULATION - LOG REDUCTION

1. Conversion raw count to colony forming units per cubic meter (cfu/m3).

- Raw count converted to positive hole corrected value using the SAS (219-Hole impactor correction table).

- Minimum reporting limit (MAL): 1000 divided by the volume of air collected by SAS (500 L) The MAL (detection limit) for the testing will be <2cfu/m^3 of air.

- Total colony forming units per cubic meter (cfu/m3): positive hole corrected count x MRL

2. Log reduction were calculated using following equation:
   Log reduction = Log (Avg of Baseline - Avg for each specified contact time)

3. All statics were calculated using MS Excel static package.

   The t-test was performed by the following

$$f = \frac{X1 - X2}{\sqrt{\frac{0^{\sigma 2} x\,1 + S'^{2}}{2}}},$$

where

- All Statistics were calculated by the MS Excel statistic package.

The data and results of log recovery are represented in Table 1 and Table 2 respectively.

RESULTS

TABLE 1: RECOVERY OF BACILLUS ATROPHAEUS AT DIFFERENT TIME INTERVALS

SAS	TIME										
	Baseline (cfu*)	Pos. hole**	cfu/m^3	1 HR (cfu)	cfu/m^3	4HR (cfu)	cfu/m^3	SHR (cfu)	cfu/m^3	24HR (cfu)	cfu/m^3
SAS 1	219	1307	2614	56	112	1	2	2	4	4	8
	208	646	1292	60	120	8	16	4	8	2	4
	219	1307	2614	32	64	2	4	3	6	4	8
SAS2	219	1307	2614	48	96	2	4	3	6	5	10
	219	1307	2614	52	104	3	6	1	2	6	12
	219	1307	2614	25	50	2	4	3	6	4	8
SAS3	219	1307	2614	72	144	5	10	1	2	4	8
	219	1307	2614	48	96	5	10	4	8	3	6
	219	1307	2614	52	104	6	12	1	2	4	8

* Positive corrected value as per the 219-hole SAS value chat

** Colony forming units

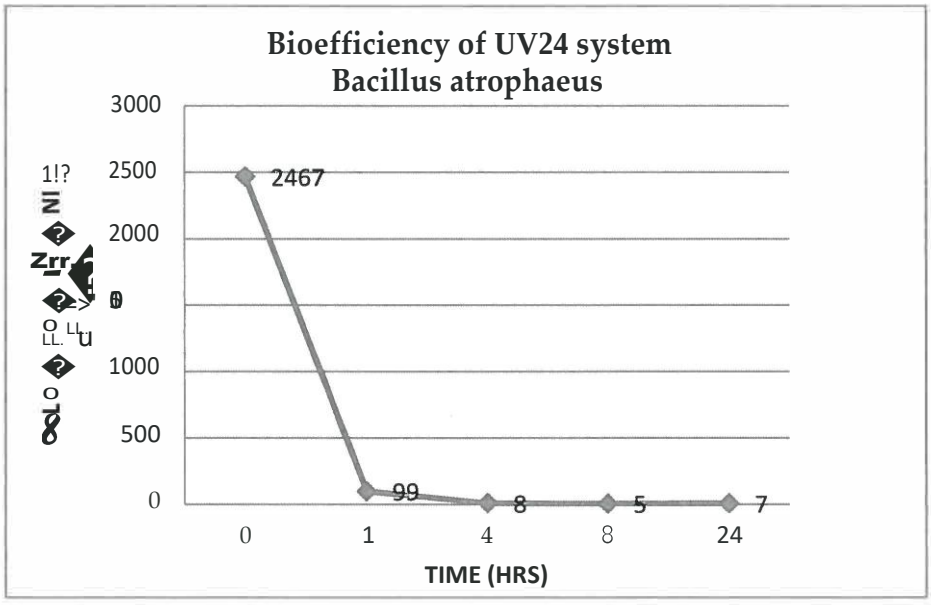


TABLE 2:

STATISTICAL ANALYSIS AND LOG REDUCTION AT EACH TIME INTERVAL FOR BACILLUS ATROPHAEUS

SAS	TIME				
	Baseline (cfu/m3)	1 HR (cfu/m3)	4 HR (cfu/m3)	8HR (cfu/m3)	24 HR (cfu/m3)
SAS 1	2614	112	2	4	8
	1292	120	16	8	4
	2614	64	4	6	8
SAS2	2614	96	4	6	10
	2614	104	6	2	12
	2614	SO	4	6	8
SAS3	2614	144	10	2	8
	2614	96	10	8	6
	2614	104	12	2	8
Average	2467	99	8	5	7
STD Dev.	73.44	22	2	3	1
Log 10	3.39	2.00	0.88	0.69	0.87
% Red		**95.9**	**99.6**	**99.7**	**99.7**
Log Red		1.40	2.51	2.70	2.53

CONCLUSION

The baseline samples showed growth of *Bacillus atrophaeus* and after the specified contact time of 1 hr there was 95.9% reduction in growth and after 4hr showed 99.6% reduction. After 8 hr and 24 hr with the UV24 system on, the samples showed 99.7% in the colony count compared to the baseline counts.

Prepared by ..... **Date** 10-29-13

Reviewed by ..... **Date** 10. 29. 13

.....

Correction Table to Adjust Colony Count from a 219-Hole Impactor Using Standard 55 mm Contact Plates and 90 mm Petri Plates

r = colony forming units count **Pr** = probable count

r	Pr	r	Pr	r	Pr	r	Pr	r	Pr	r	Pr
1	1	41	45	81	101	121	175	161	289	201	542
2	2	42	46	82	102	122	178	162	293	202	554
3	3	43	48	83	104	123	180	163	297	203	567
4	4	44	49	84	106	124	182	164	301	204	580
5	5	45	50	85	107	125	185	165	305	205	595
6	6	46	51	86	109	126	187	166	309	206	611
7	7	47	53	87	110	127	189	167	313	207	627
8	8	48	54	88	112	128	192	168	317	208	646
9	9	49	55	89	114	129	194	169	322	209	666
10	10	50	57	90	116	130	196	170	326	210	687
11	11	51	58	91	117	131	199	171	331	211	712
12	12	52	59	92	119	132	201	172	335	212	739
13	13	53	60	93	121	133	204	173	340	213	770
14	14	54	62	94	122	134	206	174	344	214	807
15	15	55	63	95	124	135	209	175	349	215	851
16	17	56	64	96	126	136	212	176	354	216	905
17	18	57	66	97	128	137	214	177	359	217	978
18	19	58	67	98	130	138	217	178	364	218	1088
19	20	59	69	99	131	139	220	179	370	219	1307
20	21	60	70	100	133	140	222	180	375		
21	22	61	71	101	135	141	225	181	381		
22	23	62	73	102	137	142	228	182	387		
23	24	63	74	103	139	143	231	183	393		
24	25	64	76	104	141	144	234	184	399		
25	26	65	77	105	142	145	237	185	405		
26	28	66	78	106	144	146	240	186	412		
27	29	67	80	107	146	147	243	187	418		
28	30	68	81	108	148	148	246	188	425		
29	31	69	83	109	150	149	249	189	432		
30	32	70	84	110	152	150	252	190	439		
31	33	71	86	111	154	151	255	191	447		
32	34	72	87	112	156	152	258	192	455		
33	36	73	88	113	158	153	261	193	463		
34	37	74	90	114	160	154	265	194	471		
35	38	75	92	115	162	155	268	195	480		
36	39	76	93	116	165	156	271	196	489		
37	40	77	95	117	167	157	275	197	499		
38	42	78	96	118	169	158	278	198	508		
39	43	79	98	119	171	159	282	199	519		
40	44	80	99	120	173	160	286	200	530		

Biological Inactivation of the UV24 System

Protocols: Biotest using UV lamp - Simulation of Field Performance

Purpose: To test the inactivation efficiency of the UV24 System during an aerosol challenge of a quantified inoculum of clinically relevant microorganisms.

Written by:

Manju Pradeep ..... **Date: 09.05.13**

Reviewed by:

Suzanne Blevins ..... **Date: 09.09.13**

Approved by: ..... **Date: 9-10-13**

Study Completion Date: 10.30.2013

1.0 Scope and Application

The UV24 System, a UV disinfection system is incorporated into a patented fluorescent ceiling fixture and is used to disinfect bacteria, viruses and fungi in hospital settings. This equipment is intended to be used in all critical care areas of the facility. The system is designed to operate quietly with 100% disinfection while delivering highly purified air. The protocol described herein determines the inactivation efficiency of the system against spore forming and vegetative bacteria which represents a wide range of applications.

2.0 Protocol Description: Surface Air Sampler (SAS) testing - Air challenge.

The UV24 System includes a High Output UV emitting lamp, a filter and a fan array which creates differential pressure to recirculate the air locally. The SAS impactor will allow bioaerosols containing microorganisms to be actively drawn into the microbial impaction sampler.

The generation of the bio aerosol will be aerosolized in a Zipwall® room with glove boxes. The generation of the bioaerosol is continuous. A defined amount of Tryptic Soy Broth containing a pre-determined inoculum is used and delivered by the nebulizer inside the Zipwall® room. Appropriate agar plates will be placed in the three SAS impactors to verify the performance of the SASes. Equipment placement as shown in Diagram 1.

The SAS will operate at the flow rate of 100 lpm for 5 minutes for a total of 500 L.

The Pro/Neb Ultra nebulizer delivers the inoculum directly into the Zipwall® room. The nebulizer is filled with 8ml of inoculum in TSB and delivers a constant rate of 7.5 lpm. The flow rate of the bioaerosol is maintained by calibrated PARI compressor. The SAS draws the mixture of air and the bio aerosol for 5 minutes and impacts on the plates.

The inoculum concentration is maintained and documented between 10"8 cfu/ml for all tests.

Before challenge:

- The Zipwall® is constructed as per manufacture instruction. The spacing of the poles is based on the movement of air in the room. The air flow is typical for commercial air space environment.
- Four glove box areas are specified in the Zipwall® room for nebulizer placement and for 3 SAS samplers.
- The UV24 unit is installed in the ceiling as per instructions and plugged in. At nominal design airflow of 50 cfm, with a MERV 6 filter and a UV lamp, the UV24 emits 15 watts of UV radiation at 253.7 nm.
- The SAS and nebulizer are decontaminated before testing.
- The room ceiling is decontaminated before testing.

Challenge:

- The air flow and the UV emittance from the AUKV24 system are continuous.
- The nebulizer is placed in one of the glove box with orifice facing towards the inside of the Zipwall® room.
- Each of the SAS samplers with appropriate agar plates is placed in the other three glove box spaces to collect the air samples.
- The ambient air is sampled before the inoculum is nebulized into the Zipwall® room. The UV24 system is turned off.
- Add 8 ml of inoculum to the sterile nebulizer. Attach the nebulizer to the connector and to the hose of the PARI compressor.
- Set timer for 15 minute. Turn on PARI compressor.
- Turn off nebulizer after 15 minute and the SAS'es are run for 5 min to collect 500 L of air as the baseline sample.
- The procedure is repeated at different time intervals of 1 hr., 4 hrs, Bhrs and 24 hrs after the UV24 system is turned on.

- The testing is done in replicates of 3 for each interval of time.
- The procedure is repeated for 3 consecutive times to get 6 sets of data for each organism at different time intervals.

After challenge:

- The impacted plates are placed in an incubator at 35◦ C, examine plates after 24 hours for growth, and count colonies. If necessary, incubate plates for an additional 24 hours. After the colonies have grown, choose countable plate. Record control counts to determine the accuracy of the challenge and the inactivation efficiency of the UV24 system.

Test organisms:

- *Serratia marcescens* ATCC14756
- *Bacillus atrophaeus* ATCC 9372

3.0 Preparation of the Inoculum/Control

1. Grow the organisms for 18-24 hours at 37°C in sterile TSB in a 5 mls sterile tube before use.
2. Turn on turbidometer and check the system using control standards.
3. Dilute 1 ml of culture grown overnight into 9 mls of sterile TSB and take 1 ml from this dilution and read the absorbance in the turbidometer. Compare the turbidity to a Mcfarland 1 standard.
4. Remove 1 ml of the diluted mixture and serially dilute to 1ff ^{8}and plate 1OOµl of this dilution onto sterile Tryptic soy agar plates for stock count verifi cation.
5. Incubate plate for 24 hours at 35°C. Count colonies to verify growth of stock culture. Multiply cfu (colony forming units) count by dilution to determine cfu/ml in diluted stock culture.
6. Make certain that biological safety cabinet blower is on and the surface is cleaned and dried with 10% bleach.

7. Operator should wear sterile gloves and gown before handling spray generator and test materials.
8. Set-up sterile materials in the BSC. Perform the testing steps in the BSC.

Inoculum

Each inoculum is prepared using basic growth methods and growth media optimal to each organism. The organisms are grown in broth and turbidity is verified using a turbidometer according to McFarland standards. Organisms are serially diluted and plated to determine viability and numeration. All organisms will be handled according to the biosafety level of which it has been assigned. Organisms will be acquired from American Type Culture Collection (ATCC) and the test strains used will not have been cultured beyond 5 passages. All bio hazardous waste will be sterilized and disposed of properly.

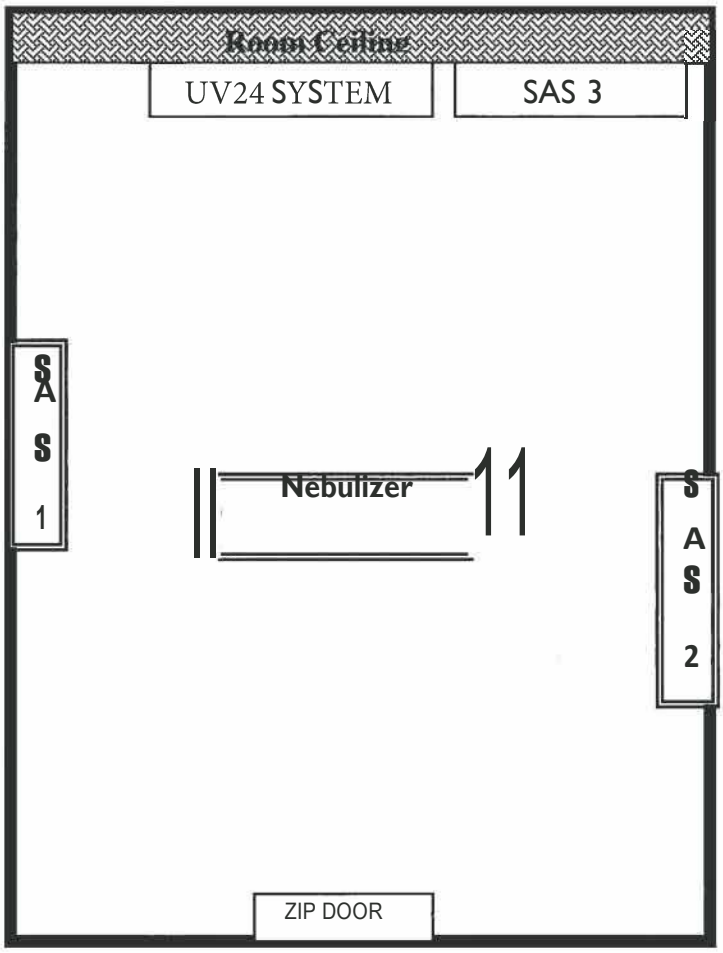
4.0 Recording and Interpreting Results:

All tests are performed according to GLP (good laboratory standards). Tests performed in triplicate. The sensitivity of the recovery method test is assumed to be 2 colony-forming units. Final numbers averaged and compared to the baseline time. Recovered organisms are counted and the colony forming units recorded. Recovery at each time interval is subtracted from recovery colony-forming units (cfu) of the baseline.

Airborne inactivation efficiency (%) = **100 (1** - **survival rate)**

Diagram 1: Air Bio test: Simulation of Field Performance

ZIPWALL® ROOM



References:

1. "Biological Inactivation Efficiency by HVAC In -Duct Ultraviolet Light Systems":
   EPA, Office of Research And Development National Homeland Security Research Center.

2. Report on the performance of the uv24 System: total ceiling solutions

   ZIPWALL® package Insert: Home Depot

3.

BIOLOGICAL INACTIVATION OF THE UV24 SYSTEM

FINAL REPORT

PROTOCOL NO. AER 09112013 Rev 3

LABORATORY PROJECT ID #13016309

Organism: *Serratia marcescens*

Contact time: 1 hr., 4hr, 8 hr. and 24 hr.

SUBMITTED BY

AEROBIOLOGY LABORATORY
43760 Trade Center Place, Suite 100
Dulles, VA 20166
Tel (703) 648-9150
Fax (703) 648-0963
e-mail: lab@aerobiology.net

BIOLOGICAL INACTIVATION OF UV24 COMPLIANCE STATEMENT

Information on the structure and activation of UV24 system resides with the sponsor of the study.

QAU STATEMENT

TITLE: Biological Inactivation of UV24 System

PROTOCOL	AER 09112013 Rev 3
LAB PROJECT	**13016309**
EVALUATION STUDY	Study I
TEST DATE	10.24.2013
DATE QAU INSPECTED	10.25.2013
DATE REPORTED	10.28.2013

STUDY OBJECTIVES

Evaluate the inactivation efficiency of the UV24 system in terms of % reduction or Log reduction of microorganism in a simulated room in the same manner as the critical care facility areas in hospital settings. The system is designed to operate quietly with 100% disinfection while delivering highly purified air. The protocol described herein determines the inactivation efficiency of the system against spore forming and vegetative bacteria which represents a wide range of applications.

The test was conducted based on the EPA protocol "Biological Inactivation Efficiency by HVAC In-Duct Ultraviolet Light Systems". The Quality Assurance Unit of Aerobiology Laboratory has inspected the lab project # 13016309 in compliance with the current GLP Regulations and the reported results accurately reflect the raw data.

INTRODUCTION

This report describes the procedure and results of the biological inactivation efficiency of UV24 system during an increased challenge of aerosoled inoculum of clinically relevant microorganisms. A medical grade nebulizer is used to deliver the accurate and consistent number of pathogens into the simulated room. The UV24 system is turned on for specified contact time during which the aerosolized microorganism comes in contact with the UV system resulting in reduction and release of purified air into the room. This test procedure is developed for a challenge level of greater than 10"8 colony forming units (CFU), which provides 1000 times higher challenge than would be expected in normal use. This method employed the fundamentals of the EPA method "Biological Inactivation Efficiency by HVAC In-Duct Ultraviolet Light Systems".

JUSTIFICATION

The surface air sampler (SAS) or impactor provides advantages over other methods for test because it can simulate inhalation of aerosoled contaminants at controlled air flow rate. The SAS pulls in 500L of air which is represented as 219 impaction holes on the media placed within the SAS. High volume of air causes multiple impactions which skews the data interpretation. The use of medical grade delivery nebulizer allows a high concentration of aerosol challenge to be aerosolized into the room. The actual count of challenging microorganism is predetermined by colony count, while the volume of the challenge aerosol is tightly controlled by monitoring the air flow and air pressure through the nebulizer. Aerosols are self-contained and they do not pose any bio-safety concerns.

DESCRIPTION OF TEST PROCEDURE

Sample conditioning and calibration

The Zipwall® is constructed as per manufacture instruction. The spacing of the poles is based on the movement of air in the room. The air flow is typical for commercial air space environment. Four glove box areas are specified in the Zipwall® room for nebulizer placement and for three SAS samplers. The UV24 is installed in the ceiling as per instructions and plugged in. At nominal design airflow of 50 cfm, with a MERV 6 filter and a UV lamp, the UV24 emits 15 watts of UV radiation at 253.7 nm. The SAS and the nebulizer are placed at the appropriate glove box. The SAS will operate at the flow rate of 100 1pm for 5 minutes for a total of 500 L.

Challenge phase

Unplug the UV24 system. Connect nebulizer to the PARI compressor. An appropriate media plate is also placed in each of the SAS. Add 8ml of inoculum to the sterile nebulizer. Attach the nebulizer to the connector and to the hose of the PARI compressor. Set timer for 15 minute. Turn on PARI compressor. The nebulizer aerosolizes the inoculum and the compressor is turned off after 15 min. The SAS are turned on for 5 min for sample collection. Three sets of samples are taken outside the simulated clean room using to SAS at 5 min to verify contamination of outside air by the indicator organism.

Contact time

The UV24 system is turned on after the baseline samples are taken. Samples are taken after the contact times of 1hr, 4 hr, 8 hr and 24 hr during which the aerosolized inoculum comes in contact with UV24 system.

Recovery phase

After sampling at specified time intervals, the plates are then placed in the incubator at 35°C for 24 hrs to recover the challenge microorganism.

Plate count *I* Result recording

Examine plates after 24 hours for growth, and colony count. If necessary, incubate plates for an additional 24 hours. After the colonies have grown, choose countable plate. Record control counts to determine the accuracy of the challenge and the inhibitory efficiency of the UV24 system. The raw colony counts are compared to the 219-hole surface air sampler (SAS) positive hole correction chart to get positive hole corrected colony count for the respective samples. (Attachment 1). The No growth sample is assumed to be <1 colony forming unit per sample and cfu/m^3 of air will be less than <2 cfu/ m^3 per analysis.

TEST MATERIALS

Testing Area (Room area)	8ft x9ft x1Oft
Equipment Source	(UV24 system) Sui1ace air
Equipment	sampler (SAS) 10.21.13
Date Received	10.24.13
Start Date	
Lab study completion date	10.26.13
Report Issue Date	10.28.13

TESTS CONDITIONS

Test Matrix	6 samples
Organism	*Serratia marcescens* ATCC 14756
Inoculum	10^8 cfu/ml
Media	Tryptic Soy Agar with 5% sheep's blood
Set up	Protocol no. 09112013 Rev 3 (Diagram 1)
Challenge Device	Nebulizer Pro NEB ULTRA II
Delivery Time	15 min
Challenge Volume	8ml
Internal Control	Pre-samples taken outside and inside the Zipwall room
[11]Base line[11]	Immediately after aerosolisation for 15 min with UV24 off 1
Contact time	hr, 4hr, 8 hr, and 24hr
Incubator	35°c
Recovery	24hr in Incubator at 35°C

CALCULATION - LOG REDUCTION

1. Conversion raw count to colony forming units per cubic meter (cfu/m3).

- Raw count converted to positive hole corrected value using the SAS (219-Hole impactor correction table).

- Minimum reporting limits (MRL): 1000 divided by the volume of air collected by SAS (500 L). The MRL (detection limit) for the testing will be <2cfu/m^3 of air.

- Total colony forming units per cubic meter (cfu/m3): positive hole corrected count x MRL

2. Log reduction were calculated using following equation:
   Log reduction= Log (Avg of Baseline - Avg for each specified contact time)

3. All statics were calculated using MS Excel static package.

   The t-test was performed by the following

$$t = \frac{X_1 - X_2}{S_{X_1 X_2} \cdot \sqrt{f7i}} \quad \text{where} \quad S_{\cdot} = \frac{Sh + S\_b}{2}$$

- All Statistics were calculated by the MS Excel statistic package.

The data and results of log recovery are represented in Table 1 and Table 2 respectively.

RESULTS

TABLE 1: RECOVERY OF SERRATIA MARCESCENS AT DIFFERENT TIME INTERVALS

SAS	TIME										
	Baseline (cfu**)	Pos. hole*	cfu/mA3	1 HR (cfu)	cfu/mA3	4HR (cfu)	cfu/mA3	SHR (cfu)	cfu/mA3	24HR (cfu)	cfu/mA3
SAS1	44	49	98	NG***	<2	NG	<2	NG	<2	NG	<2
	7	7	14	NG	<2	NG	<2	NG	<2	NG	<2
	13	13	26	NG	<2	NG	<2	NG	<2	NG	<2
SAS2	65	77	154	NG	<2	NG	<2	NG	<2	NG	<2
	5	5	10	NG	<2	NG	<2	NG	<2	NG	<2
	5	5	10	NG	<2	NG	<2	NG	<2	NG	<2
SAS3	75	92	184	NG	<2	NG	<2	NG	<2	NG	<2
	5	5	10	NG	<2	NG	<2	NG	<2	NG	<2
	2	2	4	NG	<2	NG	<2	NG	<2	NG	<2

* Positive corrected value as per the 219-hole SAS value chart

**colony forming units

*** NG assumed to be <1 cfu for raw count per plate and calculated as <2 for cfu/m3 of air.

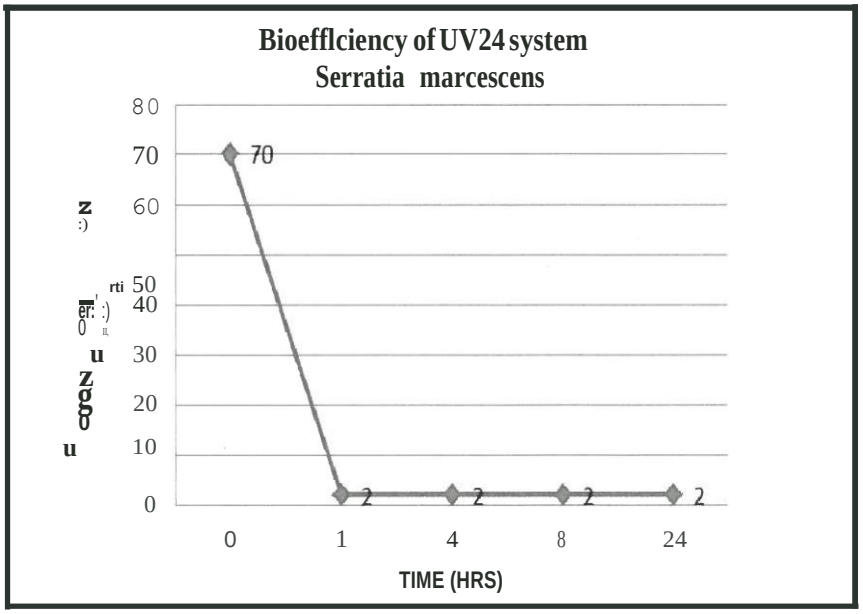


TABLE 2:

STATISTICAL ANALYSIS AND LOG REDUCTION AT EACH TIME INTERVAL FOR SERRATIA MARCESCENS

SAS	TIME				
	Baseline (cfu/m3}	1 HR (cfu/m3}	4HR (cfu/m3}	SHR (cfu/m3}	24HR (cfu/m3}
SAS1	98	2	2	2	2
	14	2	2	2	2
	26	2	2	2	2
SAS2	154	2	2	2	2
	10	2	2	2	2
	10	2	2	2	2
SAS3	184	2	2	2	2
	10	2	2	2	2
	4	2	2	2	2
Average	70	2	2	2	2
STD Dev.	66.36	0	0	0	0
Log 10	1.85	0.30	0.30	0.30	0.30
%Red		**97.0**	**97.0**	**97.0**	**97.0**

CONCLUSION

The baseline samples showed growth of *Serratia marcescens* and after the specified contact time of 1 hr., 4hr, 8 hr., and 24 hr with the UV24 system on, the samples showed complete reduction in the colony count. The Pre samples taken after 48hrs showed no *Serratia marcescens* indicating complete elimination of the indicator organism that was aerosolized into the simulated room for 15 minute.

Prepared by

Manju Pradeep ..... **Date** Jo. 9s· l3

Reviewed by

Suzanne Blevins ..... **Date** ;c>y{j./3

Correction Table to Adjust Colony Count from a 219-Hole Impactor Using Standard 55 mm Contact Plates and 90 mm Petri Plates

r = colony forming units count **Pr** = probable count

r	Pr	r	Pr	r	Pr	r	Pr	r	Pr	r	Pr
1	1	41	45	81	101	121	175	161	289	201	542
2	2	42	46	82	102	122	178	162	293	202	554
3	3	43	48	83	104	123	180	163	297	203	567
4	4	44	49	84	106	124	182	164	301	204	580
5	5	45	50	85	107	125	185	165	305	205	595
6	6	46	51	86	109	126	187	166	309	206	611
7	7	47	53	87	110	127	189	167	313	207	627
8	8	48	54	88	112	128	192	168	317	208	646
9	9	49	55	89	114	129	194	169	322	209	666
10	10	50	57	90	116	130	196	170	326	210	687
11	11	51	58	91	117	131	199	171	331	211	712
12	12	52	59	92	119	132	201	172	335	212	739
13	13	53	60	93	121	133	204	173	340	213	770
14	14	54	62	94	122	134	206	174	344	214	807
15	15	55	63	95	124	135	209	175	349	215	851
16	17	56	64	96	126	136	212	176	354	216	905
17	18	57	66	97	128	137	214	177	359	217	978
18	19	58	67	98	130	138	217	178	364	218	1088
19	20	59	69	99	131	139	220	179	370	219	1307
20	21	60	70	100	133	140	222	180	375		
21	22	61	71	101	135	141	225	181	381		
22	23	62	73	102	137	142	228	182	387		
23	24	**63**	74	103	139	143	231	183	393		
24	25	64	76	104	141	**144**	**234**	184	399		
25	26	65	77	105	**142**	145	237	185	405		
26	**28**	66	78	106	**144**	146	240	186	**412**		
27	29	67	80	107	146	147	**243**	187	**418**		
28	30	68	81	108	**148**	148	246	188	425		
29	31	69	**83**	109	150	149	249	189	**432**		
30	32	70	**84**	110	152	150	252	190	439		
31	33	71	86	111	154	151	255	191	447		
32	**34**	72	87	112	156	152	258	192	455		
33	36	73	88	113	158	153	261	193	463		
34	37	74	90	114	160	154	265	194	471		
35	38	75	92	115	162	155	268	195	480		
36	39	76	93	116	165	156	271	196	489		
37	40	77	95	117	167	157	275	197	499		
38	**42**	78	96	118	169	158	278	198	508		
39	**43**	79	98	119	171	159	282	199	519		
40	**44**	80	99	120	173	160	286	200	530		

BIOLOGICAL INACTIVATION OF THE UV24 SYSTEM

FINAL REPORT

PROTOCOL NO. AER 09112013 Rev 4

LABORATORY PROJECT ID# 13017604

Organism: *MRSA BAA 44*

Contact time: 1 hr., 4hr, 8 hr. and 24 hr.

SUBMITTED BY

AEROBIOLOGY LABORATORY
43760 Trade Center Place, Suite 100
Dulles, VA 20166
Tel (703) 648-9150
Fax (703) 648-0963
e-mail: lab@aerobiology.net

BIOLOGICAL INACTIVATION OF THE UV24 SYSTEM COMPLIANCE STATEMENT

Information on the structure and activation of UV24 system resides with the sponsor of the study.

QAU STATEMENT

TITLE: Biological Inactivation of UV24 (UV24)

PROTOCOL	AER 09112013 Rev 4
LAB PROJECT	**13017604**
EVALUATION STUDY	Study IV
TEST DATE	11.19.2013
DATE QAU INSPECTED	11.21.2013
DATE REPORTED	11.21.2013

STUDY OBJECTIVES

Evaluate the inactivation efficiency of the UV24 system in terms of % reduction or Log reduction of microorganism in a simulated room in the same manner as the critical care facility areas in hospital settings. The system is designed to operate quietly with 100% disinfection while delivering highly purified air. The protocol described herein determines the inactivation efficiency of the system against spore forming and vegetative bacteria which represents a wide range of applications.

The test was conducted based on the EPA protocol "Biological Inactivation Efficiency by HVAC In-Duct Ultraviolet Light Systems". The Quality Assurance Unit of Aerobiology Laboratory has inspected the lab project # 13017604 in compliance with the current GLP Regulations and the reported results accurately reflect the raw data.

INTRODUCTION

This report describes the procedure and results of the biological inactivation efficiency of UV24 system during an increased challenge of aerosoled inoculum of clinically relevant microorganisms. A medical grade nebulizer is used to deliver the accurate and consistent number of pathogens into the simulated room. The UV24 system is turned on for specified contact time during which the aerosolized microorganism comes in contact with the UV system resulting in reduction and release of purified air into the room. This test procedure is developed for a challenge level of greater than 10"6 colony forming units (CFU), which provides 1000 times higher challenge than would be expected in normal surrounding. This method employed the fundamentals of the EPA method "Biological Inactivation Efficiency by HVAC In-Duct Ultraviolet Light Systems".

JUSTIFICATION

The surface air sampler (SAS) or impactor provides advantages over other methods for test because it can simulate inhalation of aerosoled contaminants at controlled air flow rate. The SAS pulls in 500L of air which is represented as 219 impaction holes on the media placed within the SAS. High volume of air causes multiple impactions which skews the data interpretation. The use of medical grade delivery nebulizer allows a high concentration of aerosol challenge to be aerosolized into the room. The actual count of challenging microorganism is predetermined by colony count, while the volume of the challenge aerosol is tightly controlled by monitoring the air flow and air pressure through the nebulizer. Aerosols are self-contained and they do not pose any bio-safety concerns.

DESCRIPTION OF TEST PROCEDURE

Sample conditioning and calibration

The Zipwall® is constructed as per manufacture instruction. The spacing of the poles is based on the movement of air in the room. The air flow is typical for commercial air space environment. Four glove box areas are specified in the Zipwall® room for nebulizer placement and for three SAS samplers. The UV24 is installed in the ceiling as per instructions and plugged in. At nominal design airflow of 50 cfm, with a MERV 6 filter and a UV lamp, the UV24 emits 15 watts of UV radiation at 253.7 nm. The SAS and the nebulizer are placed at the appropriate glove box. The SAS will operate at the flow rate of 100 1pm for 5 minutes for a total of 500 L.

Challenge phase

Unplug the UV24 system. Connect nebulizer to the PARI compressor. An appropriate media plate is also placed in each of the SAS. Add 8ml of inoculum to the sterile nebulizer. Attach the nebulizer to the connector and to the hose of the PARI compressor. Set timer for 15 minute. Turn on PARI compressor. The nebulizer aerosolizes the inoculum and the compressor is turned off after 15 min. The SAS are turned on for 5 min for sample collection. Three sets of samples are taken outside the simulated clean room using to SAS at 5 min to verify contamination of outside air by the indicator organism.

Contact time

The UV24 system is turned on after the baseline samples are taken. Samples are taken after the contact times of 1hr, 4 hr, 8 hr and 24 hr during which the aerosolized inoculum comes in contact with UV24 system.

Recovery phase

After sampling at specified time intervals, the plates are then placed in the incubator at 35°C for 24 hrs to recover the challenge microorganism.

Plate count/ Result recording

Examine plates after 24 hours for growth, and colony count. If necessary, incubate plates for an additional 24 hours. After the colonies have grown., choose countable plate. Record control counts to determine the accuracy of the challenge and the inhibitory efficiency of the UV24 system. The raw colony counts are compared to the 219-hole surface air sampler (SAS) positive hole correction chart to get positive hole corrected colony count for the respective samples. (Attachment 1). The No growth sample is assumed to be <1 colony forming unit per sample and cfu/m^3 of air will be less than <2 cfu/ m^3 per analysis.

TEST MATERIALS

Testing Area (Room area)	8ft x9ft x1Oft
Equipment Source	(UV24 system) Surface air
Equipment	sampler (SAS) 10.21.13
Date Received	11.19.13
Start Date	
Lab study completion date	11.21.13
Report Issue Date	11.21.13

TESTS CONDITIONS

Test Matrix	6 samples
Organism	*MRSA BAA44*
Inoculum	10^6 cfu/ml
Media	Tryptic Soy Agar with 5% sheep blood
Set up	Protocol no. 09112013 Rev 4 (Diagram 1)
Challenge Device	Nebulizer Pro NEB ULTRA II
Delivery Time	15 min
Challenge Volume	8ml
Internal Control	Pre-samples taken outside and inside the Zipwall room
"Base line"	Immediately after aerosolisation for 15 min with UV24 off 1
Contact time	hr, 4hr, 8 hr, and 24hr
Incubator	35°c
Recovery	24hr in Incubator at 35°C

CALCULATION - LOG REDUCTION

1. Conversion raw count to colony forming units per cubic meter (cfu/m3).

- Raw count converted to positive hole corrected value using the SAS (219-Hole impactor correction table).

- Minimum reporting limits (MRL): 1000 divided by the volume of air collected by SAS (500 L). The MRL (detection limit) for the testing will be <2cfu/m^3 of air.

- Total colony forming units per cubic meter (cfu/m3): positive hole corrected count x MRL

2. Log reduction were calculated using following equation:
   Log reduction= Log (Avg of Baseline -Avg for each specified contact time)

3. All statistical analysis were calculated using MS Excel static package.

The data and results of log recovery are represented in Table 1 and Table 2 respectively.

RESULTS

TABLE 1: RECOVERY OF MRSA AT DIFFERENT TIME INTERVALS

SAS	TIME										
	Baseline (cfu*)	Pos. hole**	cfu/ m^3	1HR** (cfu)	cfu/ m^3	4HR** (cfu)	cfu/ m^3	SHR** (cfu)	cfu/ m^3	24HR** (cfu)	cfu/ m^3
SAS 1	168	317	634	3	6	1	2	1	2	1	2
	125	185	370	1	2	1	2	1	2	1	2
	.168	317	634	1	2	1	2	1	2	1	2
SAS2	219	1307	2614	1	2	1	2	1	2	1	2
	200	530	1060	2	4	1	2	1	2	1	2
	196	489	978	1	2	1	2	1	2	1	2
SAS3	176	354	708	1	2	1	2	1	2	1	2
	152	258	516	3	6	1	2	1	2	1	2
	219	1307	2614	1	2	1	2	1	2	1	2

* **Positive corrected value as per the 219-hole AS value chart**

****colony forming units**

*** **NG assumed to be <1 cfu for raw count per plate and calculated as <2 for cfu/m3 of air.**

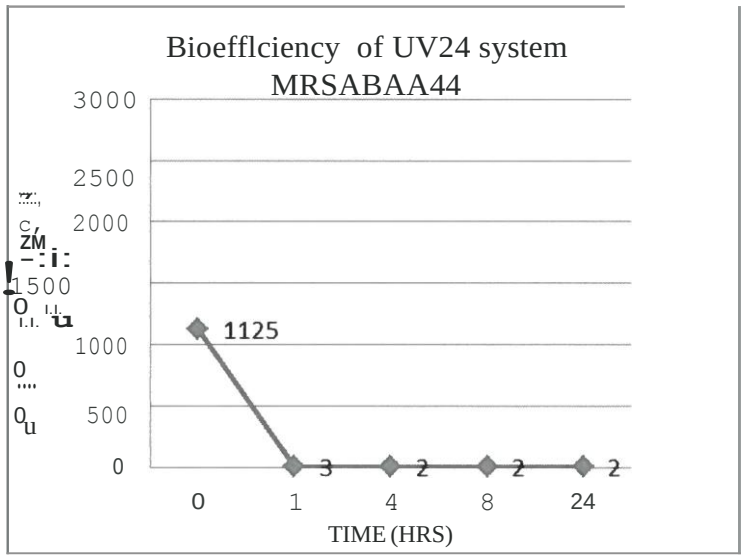


TABLE 2:

STATISTICAL ANALYSIS AND LOG REDUCTION AT EACH TIME INTERVAL FOR MRSA

SAS	TIME				
	Baseline (cfu/m3)	1 HR (cfu/m3)	4HR (cfu/m3)	8HR (cfu/m3)	24HR (cfu/m3)
SAS 1	634	6	2	2	2
	370	2	2	2	2
	634	2	2	2	2
SAS2	2614	2	2	2	2
	1060	4	2	2	2
	978	2	2	2	2
SAS3	708	2	2	2	2
	516	6	2	2	2
	2614	2	2	2	2
Average	1125	3	2	2	2
Log Red		2.56	2.75	2.75	2.75
%Red		**99.7**	**99.8**	**99.8**	**99.8**

CONCLUSION

The baseline samples showed growth of *MRSA* and after the specified contact time of 1 hr., 4hr,8 hr. and 24 hr. with the UV24 system on, the samples showed complete reduction in the colony count. The samples taken after 48hrs showed no *MRSA* indicating complete elimination of the target organism that was aerosolized into the simulated room for 15 minute.

Prepared by

Manju Pradeep . . . . . **Date** /f-Jf./3

Reviewed by

Suzanne Blevins ..St ..d. **Date** f!. er/. f"':)

BIOLOGICAL INACTIVATION OF UV24 SYSTEM

FINAL REPORT

PROTOCOL NO. AER 09112013 Rev 4

LABORATORY PROJECT ID #13016792

Organism: *Staphylococcus aureus*

Contact time: 1 hr., 4hr, 8 hr. and 24 hr.

SUBMITTED BY

AEROBIOLOGY LABORATORY
43760 Trade Center Place, Suite 100
Dulles, VA 20166
Tel (703) 648-9150
Fax (703) 648-0963
e-mail: lab@aerobiology.net

BIOLOGICAL INACTIVATION OF UV24 SYSTEM COMPLIANCE STATEMENT

Information on the structure and activation of UV24 system resides with the sponsor of the study.

QAU STATEMENT

TITLE: Biological Inactivation of UV24 (UV24)

PROTOCOL	AER 09112013 Rev 4
LAB PROJECT	**13016792**
EVALUATION STUDY	Study Ill
TEST DATE	11.12.2013
DATE QAU INSPECTED	11.14.2013
DATE REPORTED	11.15.2013

STUDY OBJECTIVES

Evaluate the inactivation efficiency of the UV24 system in terms of % reduction or Log reduction of microorganism in a simulated room in the same manner as the critical care facility areas in hospital settings. The system is designed to operate quietly with 100% disinfection while delivering highly purified air. The protocol described herein determines the inactivation efficiency of the system against spore forming and vegetative bacteria which represents a wide range of applications. .

The test was conducted based on the EPA protocol "Biological Inactivation Efficiency by HVAC In-Duct Ultraviolet Light Systems". The Quality Assurance Unit of Aerobiology Laboratory has inspected the lab project # 13016792 in compliance with the current GLP Regulations and the reported results accurately reflect the raw data.

INTRODUCTION

This report describes the procedure and results of the biological inactivation efficiency of UV24 system during an increased challenge of aerosoled inoculum of clinically relevant microorganisms. A medical grade nebulizer is used to deliver the accurate and consistent number of pathogens into the simulated room. The UV24 system is turned on for specified contact time during which the aerosolized microorganism comes in contact with the UV system resulting in reduction and release of purified air into the room. This test procedure is developed for a challenge level of greater than 10"6 colony forming units (CFU), which provides 1000 times higher challenge than would be expected in normal use. This method employed the fundamentals of the EPA method "Biological Inactivation Efficiency by HVAC In-Duct Ultraviolet Light Systems".

JUSTIFICATION

The surface air sampler (SAS) or impactor provides advantages over other methods for test because it can simulate inhalation of aerosoled contaminants at controlled air flow rate. The SAS pulls in SOOL of air which is represented as 219 impaction holes on the media placed within the SAS. High volume of air causes multiple impactions which skews the data interpretation. The use of medical grade delivery nebulizer allows a high concentration of aerosol challenge to be aerosolized into the room. The actual count of challenging microorganism is predetermined by colony count, while the volume of the challenge aerosol is tightly controlled by monitoring the air flow and air pressure through the nebulizer. Aerosols are self-contained and they do not pose any bio-safety concerns.

DESCRIPTION OF TEST PROCEDURE

Sample conditioning and calibration

The Zipwall® is constructed as per manufacture instruction. The spacing of the poles is based on the movement of air in the room. The air flow is typical for commercial air space environment. Four glove box areas are specified in the Zipwall® room for nebulizer placement and for three SAS samplers. The UV24 is installed in the ceiling as per instructions and plugged in. At nominal design airflow of 50 cfm, with a MERV 6 filter and a UV lamp, the UV24 emits 15 watts of UV radiation at 253.7 nm. The SAS and the nebulizer are placed at the appropriate glove box. The SAS will operate at the flow rate of 100 1pm for 5 minutes for a total of 500 L.

Challenge phase

Unplug the UV24 system. Connect nebulizer to the PARI compressor. An appropriate media plate is also placed in each of the SAS. Add 8ml of inoculum to the sterile nebulizer. Attach the nebulizer to the connector and to the hose of the PARI compressor. Set timer for 15 minute. Turn on PARI compressor. The nebulizer aerosolizes the inoculum and the compressor is turned off after 15 min. The SAS are turned on for 5 min for sample collection. Three sets of samples are taken outside the simulated clean room using to SAS at 5 min to verify contamination of outside air by the indicator organism.

Contact time

The UV24 system is turned on after the baseline samples are taken. Samples are taken after the contact times of 1hr, 4 hr, 8 hr and 24 hr during which the aerosolized inoculum comes in contact with UV24 system.

Recovery phase

After sampling at specified time intervals, the plates are then placed in the incubator at 35°C for 24 hrs to recover the challenge microorganism.

Plate count / Result recording

Examine plates after 24 hours for growth, and colony count. If necessary, incubate plates for an additional 24 hours. After the colonies have grown, choose countable plate. Record control counts to determine the accuracy of the challenge and the inhibitory efficiency of the UV24 system. The raw colony counts are compared to the 219-hole surface air sampler (SAS) positive hole correction chart to get positive hole corrected colony count for the respective samples. (Attachment 1). The No growth sample is assumed to be <1 colony forming unit per sample and cfu/m^3 of air will be less than <2 cfu/ m^3 per analysis.

TEST MATERIALS

Testing Area (Room area)	8ft x9ft x1Oft
Equipment Source	(UV24 system) Surface air
Equipment	sampler (SAS) 10.21.13
Date Received	11.12.13
Start Date	
Lab study completion date	11.14.13
Report Issue Date	11.15.13

TESTS CONDITIONS

Test Matrix	6 samples
Organism	*Staphylococcus aureus ATCC 6538*
Inoculum	10^6 cfu/ml
Media	Tryptic Soy Agar with 5% sheep blood
Set up	Protocol no. 09112013 Rev 4 (Diagram 1)
Challenge Device	Nebulizer Pro NEB ULTRA II
Delivery Time	15 min
Challenge Volume	8ml
Internal Control	Pre-samples taken outside and inside the Zipwall room
"Base line"	Immediately after aerosolisation for 15 min with UV24 off
Contact time	1 hr, 4hr, 8 hr, and 24hr
Incubator	35°C
Recovery	24hr in Incubator at 35°C

CALCULATION- LOG REDUCTION

1. Conversion raw count to colony forming units per cubic meter (cfu/m3).

- Raw count converted to positive hole corrected value using the SAS (219-Hole impactor correction table).

- Minimum reporting limits (MRL): 1000 divided by the volume of air collected by SAS (500 L). The MRL (detection limit) for the testing will be <2cfu/m^3 of air.

- Total colony forming units per cubic meter (cfu/m3): positive hole corrected count x MRL

2. Log reduction were calculated using following equation:
   Log reduction= Log (Avg of Baseline -Avg for each specified contact time)

3. All statistical analysis were calculated using MS Excel static package.

The data and results of log recovery are represented in Table 1 and Table 2 respectively.

RESULTS

TABLE 1: RECOVERY OF STAPHYLOCOCCUSAUREUS AT DIFFERENT TIME INTERVALS

SAS	TIME										
	Base line (cfu*)	Pa s . ho le **	cfu/ m^3	1 HR** (cfu)	cfu/ m^3	4 HR ** (cfu)	cfu/ m^3	8 HR ** (cfu)	cf u/ m^3	24HR** {cfu)	cfu/ m^3
SAS 1	219	1307	2614	30	60	31	62	4	8	1	2
	219	1307	2614	15	30	31	62	15	30	1	2
	219	1307	2614	**44**	**88**	29	58	34	68	1	2
SAS 2	219	1307	2614	49	98	10	20	1	2	1	2
	219	1307	2614	39	78	3	6	3	6	1	2
	219	1307	2614	54	108	19	38	1	2	1	2
SAS 3	219	1307	2614	54	108	12	24	1	2	1	2
	219	1307	2614	19	38	25	50	1	2	1	2
	219	1307	2614	28	56	39	78	2	4	1	2

* Positive corrected value as per the z19-hole SAI **value chart**

**colony forming units

*** NG assumed to be <1 cfu for raw count per plate and calculated as <2 for cfu/m3 of air.

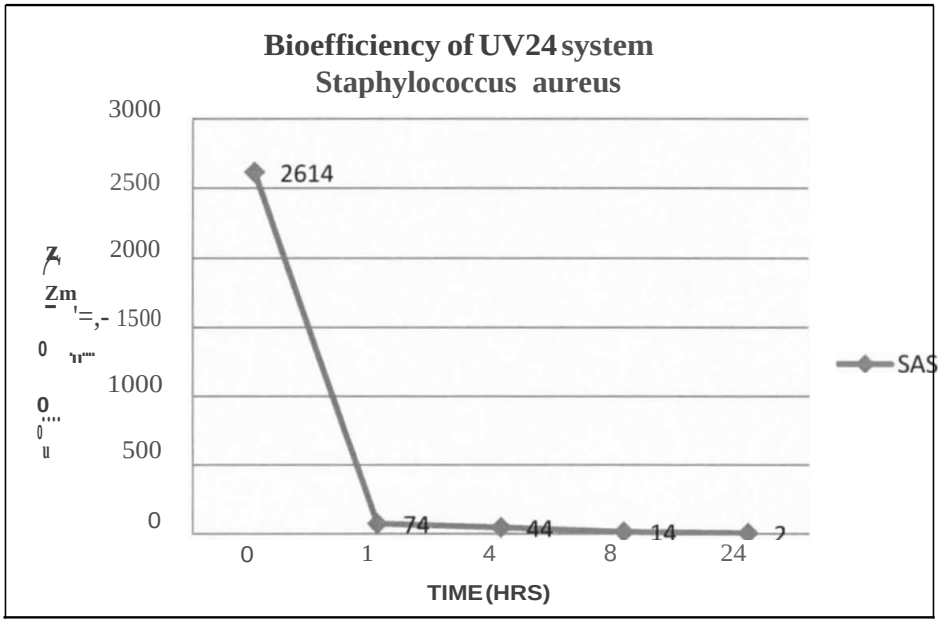


TABLE 2:

STATIST ICAL ANALYSIS AND LOG REDUCTION AT EACH TIME INTERVAL FOR STAPHYLOCOCCUS AUREUS

SAS	TIME				
	Baseline (cfu/m3)	1 HR (cfu/m3)	4HR (cfu/m3)	SHR (cfu/m3)	24HR (cfu/m3)
SAS 1	2614	60	62	8	2
	2614	30	62	30	2
	2614	88	58	68	2
SAS2	2614	98	20	2	2
	2614	78	6	6	2
	2614	108	38	2	2
SAS3	2614	108	24	2	2
	2614	38	SO	2	2
	2614	56	78	4	2
Average	2614	74	44	14	2
Log Red		1.55	1.77	2.28	3.12
%Red		**97.2**	**98.3**	**99.5**	**99.9**

CONCLUSION

The baseline samples showed growth of *Staphylococcus aureus* and after the specified contact time of 1 hr., 4hr, and 8 hr. with the UV24 system on, the samples showed gradual reduction in the colony count. After 24 hrs, there was complete reduction in the colony count. The samples taken after 48hrs showed no *Staphylococcus aureus* indicating complete elimination of the target organism that was aerosolized into the simulated room for 15 minute.

Prepared by

Manju Pradeep ..... **Date** 11.(S·13

Reviewed by

Suzanne Blevins  **Date** ((, /- 13

Predicted Value of the UV D90 for MERS Virus and Performance of the UV24 Unit

Prepared by Dr. Wladyslaw Kowalski
On 05-13-14

Executive Summary

The predicted D90 value for MERS Virus is **13.7 J/m^2**, with a 95% confidence interval between J/m^2. This prediction is based on genomic analysis of the NCBI genome for MERS Coronavirus, genome number NC_019843. The input data and analysis results are shown on the following pages.

The predicted D90 is for water applications but the values are conservative to use for air and surface disinfection applications. Details on the genomic model are available from Kowalski 2009, 2009a, 2009b, 2009c, 2009d, & 2011). The specific genomic model used in this analysis is as yet unpublished, but is similar to the previously published models and is slightly more accurate. Analysis was performed using the Aerobiological Engineering proprietary program GSA (Genomic Sequence Analyzer) 1.0.1, Copyright 2014.

Also included is an evaluation of the performance for the UV24 unit against the MERS virus. This evaluation indicates the MERS virus will be eradicated at a very high rate and will be virtually sterilized from the UV24 airstream in a single pass.

1.1 Single Stranded RNA Viruses

The Middle Eastern Respiratory virus is an ssRNA virus and has no known measured UV rate constant or D90 value. The complete genome (NC_019843) was analyzed and compared to the existing ssRNA virus model. The genomic model for all ssRNA viruses is shown in Figure 1.1, where it can be seen that the r^2 value for the curve fit of the model is 94.09%. The relative dimerization value, Dv, for MERS virus is 0.055858, which plots out on Figure 1.1 to be about 13.7 J/m^2. Appendix A summarizes the input and output data for the genomic model of MERS Coronavirus.

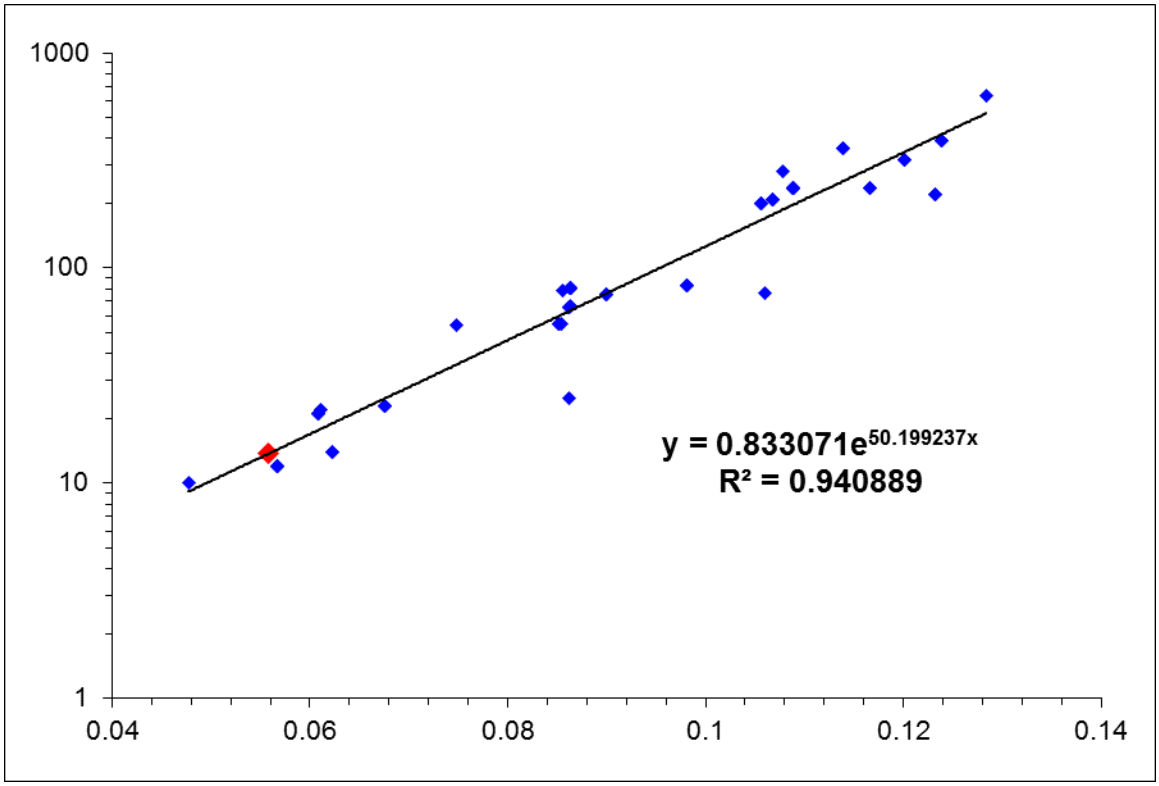


Figure 1.1: Genomic model for 27 ssRNA viruses (62 data sets). Based on Kowalski et al 2014 (unpublished). Red dot indicates the location of MERS Virus.

Figure 1.2 shows the estimates of the upper and lower confidence interval for 95% of the data. The 95% confidence interval is 6.3-20 J/m^2. The maximum value, 20 J/m^2 is suggested as a conservative value to use for design purposes.

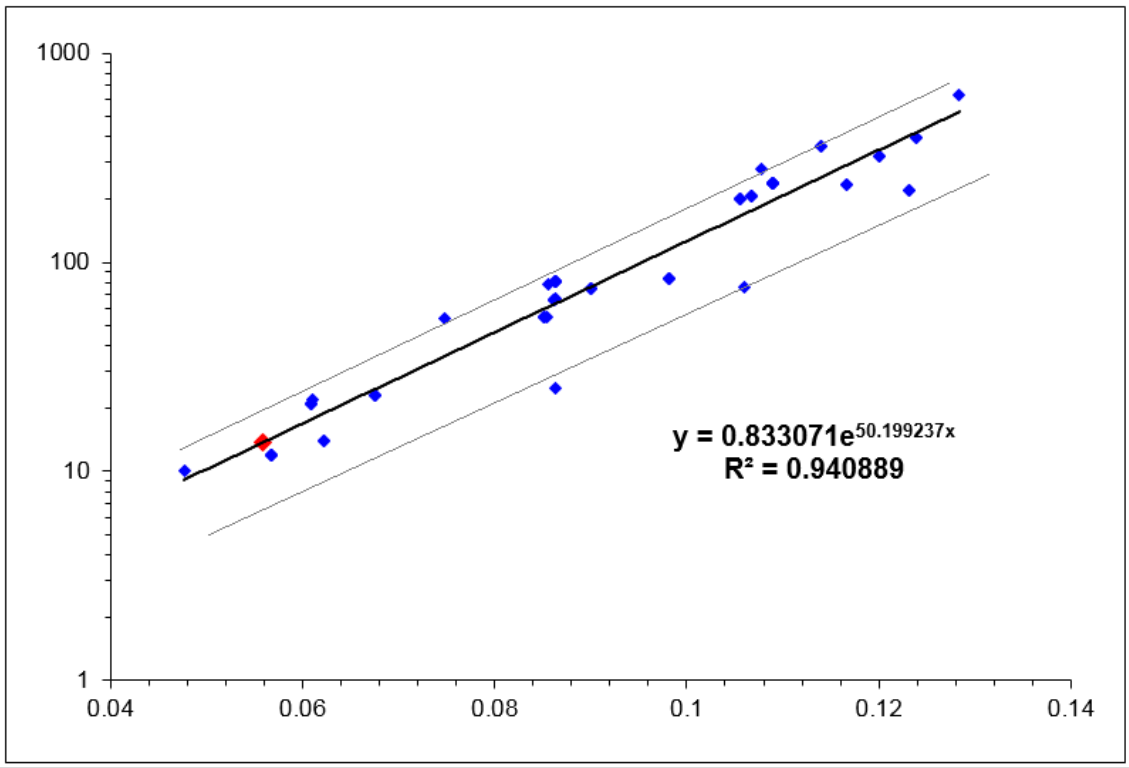


Figure 1.2: 95% confidence interval range for 62 data sets. Upper limit is 20 J/m^2, and lower limit is 6.3 J/m^2.

2.1 Performance of the UV24 Against MERS Virus

The UV24 system has two components, an ultraviolet light and a MERV 6 filter. The performance curve for a typical MERV 6 filter is shown in Figure 2.1, with the curve extended via modeling into the virus size range. MERS Coronavirus sits within the Most Penetrating Particle (MPP) size range of this, and all other MERV filters. The removal rate by the MERV 6 filter is seen to be approximately 0.05 or 5%.

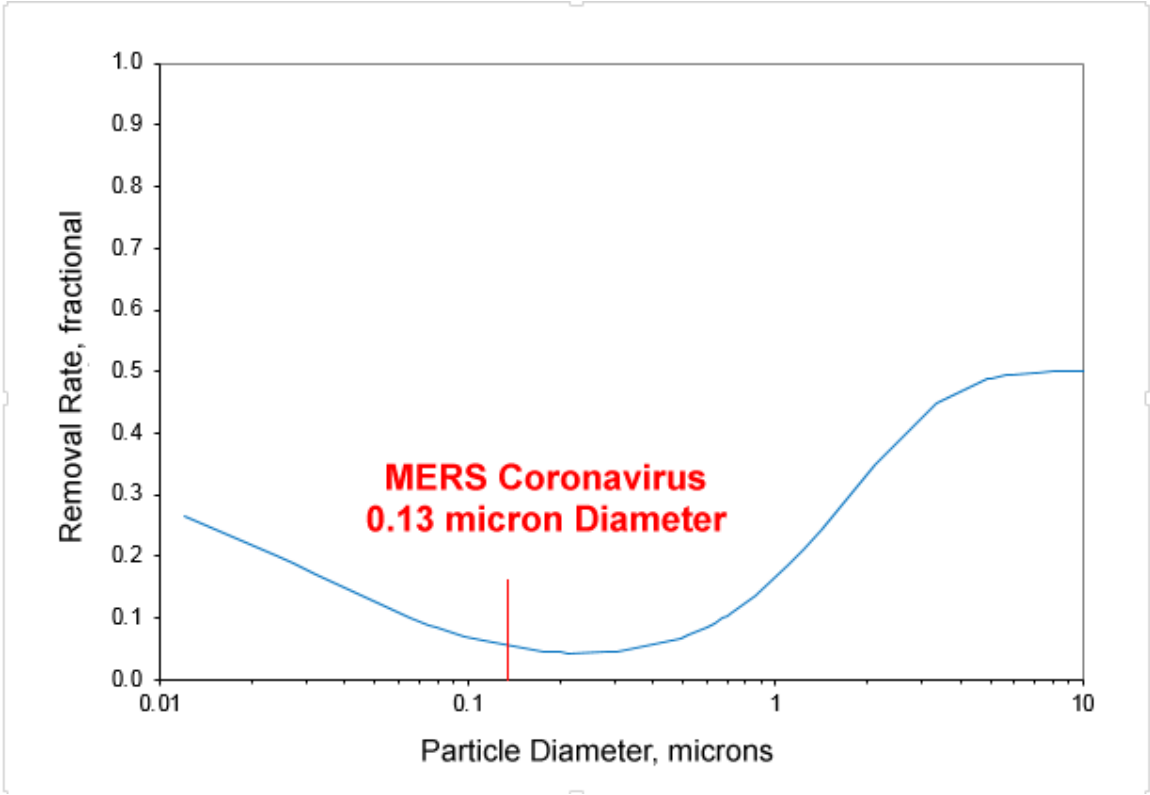


Figure 2.1: Filter Performance Curve for a Typical MERV 6 Filter, extended into the virus size range (Kowalski 2009). MERS Coronavirus is shown to be within the Most Penetrating Particle size range.

The UV component of the UV24 has an established UV dose of 198 J/m^2. The D90 dose for MERS Coronavirus is 13.7 J/m^2. This converts to a first stage UV rate constant of

$$k1 = - \ln(0.10)/D90 = 0.16807 \text{ m}^2/\text{J}$$

The virus survival at 198 J/m^2 is then computed to be

$$S = \exp(-k\ 198) = 3.527 \times 10^{-15}$$

The inactivation rate is seen, therefore, to approach zero, and to be well over six logs of reduction, or virtual sterility. Figure 2.2 shows the survival curve

of MERS virus when exposed to ultraviolet radiation. Based on this evaluation, the UV24 will produce over six logs of reduction of any airborne MERS virus.

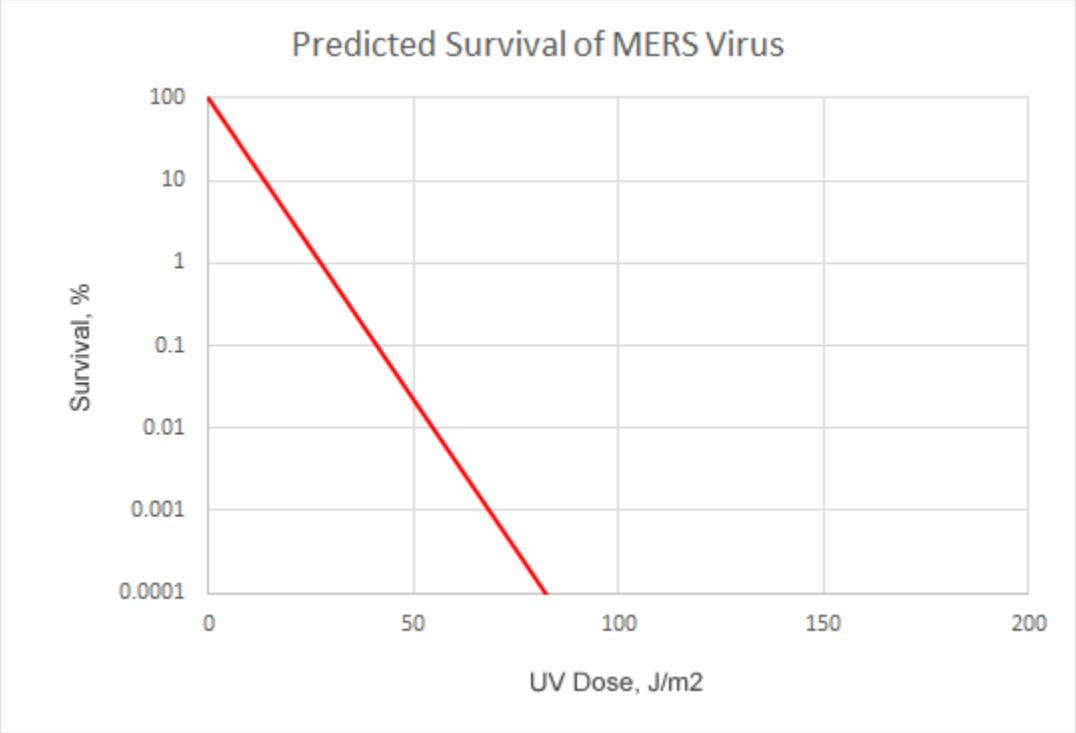


Figure 2.2: Predicted Survival of MERS Virus under UV exposure.

3.0 References

Kowalski, W. J. (2009). *Ultraviolet Germicidal Irradiation Handbook: UVGI for Air and Surface Disinfection.* Springer, New York.

Kowalski, W., Bahnfleth, W., and Hernandez, M. (2009). "A Genomic Model for Predicting the Ultraviolet Susceptibility of Bacteria and Viruses." *IUVA*, Amsterdam

Kowalski, W., Bahnfleth, W., and Hernandez, M. (2009a). "A Genomic Model for the Prediction of Ultraviolet Inactivation Rate Constants for RNA and DNA Viruses." *IUVA*, Boston, MA

Kowalski, W. J. (2009b). "Genomic Modeling of Ultraviolet Susceptibility for Viruses and Bacteria." *(unpublished manuscript)*

Kowalski, W., Bahnfleth, W., and Hernandez, M. (2009c). "A Genomic Model for Predicting the Ultraviolet Susceptibility of Viruses." *IUVA News* 11(2), 15-28.

Appendix A: Genomic Parameters for MERS Virus

Name: MERS
RefSeq: NC_019843.2
Type: Single Stranded DNA
Strand: Template

Size: 30111 bp
T: 9815
A: 7897
C: 6096
G: 6303
GC: 12399
TA: 17712
Y: 15911
R: 14200

TT Pairs: 5326
TC Pairs: 3404
CT Pairs: 4606
CC Pairs: 2082
YR Pairs: 7966
Total Pairs: 23384
S: 16231

TT Hyperprimers: 1221.201672
TC Hyperprimers: 1008.970165
CT Hyperprimers: 1208.451832
CC Hyperprimers: 514.5694284
YR Hyperprimers: 2368.0
Total Hyperprimers: 6321.4568

Clusters: 2805
Mean Cluster Size: 5.784390591589451
==========

Hyperprimer Lookup Values:

Hy	Hc
1	0.0
2	0.0
3	0.5
4	0.75
5	0.88
6	0.983023614611054
7	0.994644615260238
8	1.0