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AEMTEK ANALYTICAL REPORT

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Reported to: Li Zhang, Application Engineer, RayVio, Inc., 3980 Trust Way, Hayward, CA 94545
lzhang@rayvio.com
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Surface Antimicrobial Efficacy Study

Introduction

The objectives of this antimicrobial efficacy study were to evaluate the ability of RayVio's UV LED system technology to inactivate pathogens important in hospital and food applications when inoculated onto test matrices. The organisms included in this study were *Salmonella, Listeria monocytogenes* and Methicillin-resistant *Staphylococcus aureus (*MRSA). The inactivation of the pathogens of interest were evaluated under different drive current and treatment time conditions, as determined by RayVio. Treatment of the inoculated surfaces using UV LED was performed by RayVio's personnel while testing was performed by AEMTEK personnel, both activities were carried out at AEMTEK laboratory facility in Fremont, California.

Materials & Methods

The test organisms consisted of *Salmonella* Thyphimurium ATCC 14028, *Salmonella* Montevideo ATCC 8387, *Listeria monocytogenes* ATCC 19111, *Listeria monocytogenes* ATCC 19114 and Methicillin-resistant *Staphylococcus aureus* ATCC 43300. Each test organism was cultured separately through successive passes in nutrient broth. The strains were enumerated with dilutions in phosphate buffered saline (PBS) and plated onto XLD agar for *Salmonella*, HardyCHROM™ *Listeria* Agar for *Listeria* and Tryptic Soy Agar (TSA) for *S. aureus.* Plates were incubated at 35°C for 24-48 hours. A separate cocktail for *Salmonella* and *Listeria* was prepared on the day of the study and used as the liquid inoculum.

The RayVio UV LED system, consisting of a top emitter that emits UV light and provides surface disinfection capabilities, was setup per recommended specifications by RayVio. Sterile stainless steel coupons were inoculated with the appropriate bacteria at 6 logs or higher in order to determine the inactivation capabilities of the UV LED system following exposure. The inoculation area on the coupons was 1 sq. in. The UV LED light system was placed 10 mm above the inoculated coupon and the appropriate drive current and treatment time was employed for each bacterium,

AEMTEK, INC.
46309 Warm Springs Blvd., Fremont, CA 94539
Phone: 510-979-1979 Fax: 510-668-1980
Email: lab@aemtek.com Website: www.aemtek.com

with three replicates of treatment and testing performed for each bacterium and treatment combination. An untreated control for all organisms was also included in the study.

Immediately following the UV LED treatment, the inoculated 1 sq. in. on the stainless steel coupon was swabbed and neutralized in 1mL buffer. Appropriate dilutions were made in PBS, and samples were plated onto the appropriate agar, XLD for *Salmonella*, HardyCHROM™ *Listeria* Agar for *Listeria* and Tryptic Soy Agar (TSA) for *S. aureus.* Plates were incubated at 35°C for 24 - 48 hours and counts recorded as CFU/sq. in.

Results

The controls for all three organisms, *Listeria monocytogenes, Salmonella* and MRSA were all in the targeted 6 log range. Following the initial set of treatment configurations, as listed in Table 1 below, all test configurations had reduced the inoculated organisms to around 10 CFU/sq. in. or below. This was a reduction of between 5 and 6 logs for all of the test configurations and organisms.

Following these results, a second set of surface efficacy trials were completed with *Salmonella* at lower drive currents and treatment times. This data, shown in Table 2, again demonstrated large reductions in *Salmonella* counts as compared to the control, even at the lower drive current and treatment times. Log reductions were between 4 and 5 logs for all test configurations.

Table 1: Surface Efficacy Test Configuration Results

LED bank	Organism	Drive Current [mA]	Treatment Time [sec]	Treatment (CFU/sq. in.)	Duplicate (CFU/sq. in.)	Triplicate (CFU/sq. in.)	Average (CFU/sq. in.)	Untreated Control (CFU/sq. in.)
Surface	Listeria	50	40	2.0E+01	< 10	< 10	1.0E+01	3.3E+06
Surface	Listeria	50	80	< 10	1.0E+01	2.0E+01	1.0E+01	
Surface	Listeria	50	120	1.0E+01	1.0E+01	1.0E+01	1.0E+01	
Surface	MRSA	50	40	4.0E+01	< 10	< 10	1.0E+01	6.4E+06
Surface	MRSA	50	80	< 10	<10	< 10	< 10	
Surface	MRSA	50	120	1.0E+01	< 10	2.0E+01	1.0E+01	
Surface	Salmonella	50	40	2.0E+01	1.0E+01	2.0E+01	2.0E+01	2.4E+06
Surface	Salmonella	50	80	< 10	1.0E+01	1.0E+01	1.0E+01	
Surface	Salmonella	50	120	2.0E+01	< 10	1.0E+01	1.0E+01	
Surface	Salmonella	150	40	< 10	< 10	< 10	< 10	

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Table 2: Surface Efficacy Follow-up Test Configuration Results

LED bank	Organism	Drive Current [mA]	Treatment Time [sec]	Treatment (CFU/sq. in.)	Duplicate (CFU/sq. in.)	Triplicate (CFU/sq. in.)	Average (CFU/sq. in.)	Untreated Control (CFU/sq. in.)
Surface	Salmonella	50	20	5.0E+01	1.0E+02	3.0E+01	6.0E+01	5.4E+06
Surface	Salmonella	50	40	5.0E+01	1.0E+01	4.0E+01	3.3E+01	
Surface	Salmonella	25	10	6.4E+02	2.2E+02	3.1E+02	3.9E+02	
Surface	Salmonella	25	20	1.9E+02	1.3E+02	2.0E+02	1.7E+02	

Conclusions

The test configurations included in this study, consisting of drive current and treatment time, were effective at reducing levels of all organisms tested 4 logs or greater. The higher drive current and treatment times, which were tested against *Listeria monocytogenes, Salmonella* and Methicillin-resistant *Staphylococcus aureus*, were able to reduce bacteria levels by 5 to 6 logs. The lower drive current and treatment time combinations tested in the follow-up study were still effective against *Salmonella*, with reductions of 4 to 5 logs.

Reported by,

Heidi Wright, Ph.D.
Director of Research
AEMTEK, Inc.

Florence Wu, Ph.D.
President
AEMTEK, Inc.

AEMTEK, INC.
46309 Warm Springs Blvd., Fremont, CA 94539
Phone: 510-979-1979 Fax: 510-668-1980
Email: lab@aemtek.com Website: www.aemtek.com