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FINAL REPORT

AL Systems Ultraviolet Germicidal Irradiation Efficacy

2020-RES-044

Sponsor/Product Owner:
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217 Yale Avenue
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Test Facility:
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SIGNATURES:

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Table of Contents

1. Purpose

This study demonstrated the ultraviolet germicidal irradiation efficacy of the MDS/ADS UV (X)20 family of devices box by evaluating the antimicrobial efficacy on eyeglass frames. This study did not attempt to determine the ultraviolet dose delivered by the MDS/ADS UV (X)20 family of devices or employ the use of a test soil.

2. References

2.1. AAMI TIR No. 12:2010 *Designing, Testing, and Labeling Reusable Medical Devices for Reprocessing in Health Care Facilities: A Guide for Medical Device Manufacturers*

2.2. ASTM E3135-18 *Standard Practice for Determining Antimicrobial Efficacy of Ultraviolet Germicidal Irradiation Against Microorganisms on Carriers with Simulated Soil*

2.3. iuvo work instruction V-3 *Sterilization Validation of Reusable Medical Devices*

3. Test System Justification

The test system used both AAMI TIR No. 12: 2010 *Designing, Testing, and Labeling Reusable Medical Devices for Reprocessing in Health Care Facilities: A Guide for Medical Device Manufacturers* and ASTM E3135-18 *Standard Practice for Determining Antimicrobial Efficacy of Ultraviolet Germicidal Irradiation Against Microorganisms on Carriers with Simulated Soil* as guidance.

4. Responsibilities

4.1. iuvo BioScience, Rush, NY

- 4.1.1. Preparation and approval of protocol
- 4.1.2. Execution of protocol
- 4.1.3. Preparation of report
- 4.1.4. Record retention

4.2. AL Systems

- 4.2.1. Provide test articles for inoculation
- 4.2.2. Review and approval of protocol
- 4.2.3. Review and approval of report

5. Definitions

5.1. AAMI – Association for the Advancement of Medical Instrumentation

5.2. ASTM – American Society for Testing and Materials

5.3. ATCC – American Type Culture Collection

5.4. CFU – Colony Forming Units

5.5. UV – Ultraviolet

5.6. UVGI – Ultraviolet Germicidal Irradiation

6. Materials and Equipment

Materials used may include but not be limited to the following:

70% Sterile Isopropyl Alcohol (IPA)
PO_4 Buffer w/ 0.1% Tween
Fetal Bovine Serum (FBS)
Sterile Saline
Sterile H_20
Sterile Nitrile Gloves
Sterile Purified Water
Sterile Lint-Free Cloth
Trypticase Soy Agar (TSA)
Orbital Shaker and/or Sonicator
UV/Vis Spectrophotometer
Sterile pipette tips
Calibrated micropipettors

7. Test Article Description

The MDS UV 420 is a representative of the ADS/MDS UV X20 family of devices. ADS/MDS UV X20 devices are manually or semi-automated devices operating in the UV spectrum at a peak of 254 nm. Devices in the family have a total power rating of 5 to 16 watts (W). All devices regardless of configuration maintain fluence level of 0.005 W/cm^2 *sec, providing a total exposure of 40 seconds or 0.2 (W * sec)/cm^2. Test article specifications were supplied by sponsor.

8. Challenge Organisms

Staphylococcus aureus ATCC #6538

Escherichia coli ATCC # 8739

9. Controls

9.1. Preparation of Inoculum

Cultures were maintained in the manner recommended by the curator of the culture collection. Cultures were no greater than five (5) passes removed from the ATCC culture depository.

Staphylococcus aureus and *Escherichia coli* were prepared using the following procedure:

1. Challenge organism was aseptically transferred onto individual fresh TSA plates and incubated for 18-24 hours at 30-35°C.
2. Subsequent to incubation, the bacteria were harvested using sterile DPBST with a sterile cotton swab to dislodge the organisms from the agar surface.
3. The resulting suspension was washed using centrifugation at 20-25°C for 10 minutes at ≤4000 x g.
4. After washing, the supernatant was decanted, the pellet resuspended in fresh sterile diluent and the suspension washed a second time.
5. Following the second wash, the pellet was resuspended in fresh sterile diluent.
6. Challenge organism suspension was spectrophotometrically adjusted in sterile diluent to a concentration of approximately $1.0x10^8$-$1.0x10^9$ CFU/mL.
7. After spectrophotometric adjustment, suspension was centrifuged at 20-25°C for 10 minutes at ≤4000 x g. The supernatant was decanted, and the pellet resuspended in %5 FBS.
8. *S. aureus* and *E. coli* suspensions were used on the day of preparation.

9.2. Validation of Recovery Methods

The recovery methods were validated independently for each of two challenge organisms. The inoculation site was inoculated with ≤ 150 CFU of the challenge organism and allowed to dry for 10 minutes. Recovery was performed as described in the recovery section of this protocol 10.4. The TSA plates were incubated at 30-35°C for 3 days. A successful recovery of 50% or greater of the inoculated challenge organism population was demonstrated for both challenge organisms.

9.3. Positive Controls

Three (3) carriers (eyeglass frames) were inoculated with the same volume of suspension that is applied to each test carriers as described in section 10.2. The positive control carriers were not subjected to UV treatment. The untreated carriers were recovered and enumerated using the methods described in sections 10.4 and 10.5.

9.4. Inoculum Population Controls

The population of the inoculum was determined using techniques described in section 10.5. TSA plates were incubated at 30-35°C for 3 days prior to enumeration.

9.5. Negative Controls

Three (3) uninoculated carriers (eyeglass frames) were processed in the same way as the test carriers. Recovery and enumeration was performed as described in sections 10.4 and 10.5.

10. Procedures

10.1. General

The challenge site indicated by the sponsor was an area on eyeglass frames that could likely introduce pathogens to the eye during normal use. The test articles were packaged in sponsor supplied sterilized PET sealable bags. The test carriers were then exposed to UVGI in an MDS UV 420 device. The test carriers were processed in four groups. Groups 1 and 2 were the test groups. Group 1 were subjected to one UVGI exposure in the test device, while Group 2 were subjected to two UVGI exposures in the test device. Group 3 were the positive control carriers that were inoculated but not subjected to UVGI. Group 4 were the negative control group that was not inoculated but was subjected one UVGI exposure in the test device. Note: carriers were inoculated with a single challenge organism resulting in two sets of Groups 1, 2 and 3.

10.2. Inoculation

Two groups of test carriers and one group of positive control carriers (3 carriers in each group) were challenged with a $\geq 10^6$ level of challenge organism at the following location: See **Attachment I** for picture of the challenge site.

1. The inside of the lens that would face the eye of the patient wearing the glasses.

E. coli and *S. aureus* inoculations were allowed to dry for 10 minutes at ambient temperature.

To confirm the population of the suspensions used to inoculate the test carriers, the same volume of the suspensions were plated in duplicate, using serial dilutions as needed, over poured with TSA and incubated at 30-35 °C for a 3 days.

10.3. Conduct of UVGI Treatment

The carrier groups were exposed to UVGI in the MDS UV 420 device as follows:

Group	Treatment	UVGI Exposure	Approx. Exposure Duration
Group 1 (test carrier group 1)	Inoculated	One exposure cycle	40-45 seconds
Group 2 (test carrier group 2)	Inoculated	Two exposure cycles	80-90 seconds
Group 3 (positive controls)	Inoculated	No exposure	N/A
Group 4 (negative controls)	Not inoculated	One exposure cycle	40-45 seconds

After the test cycle, each carrier was aseptically transferred to the extraction container.

10.4. Recovery

Recovery was performed using agitation. Devices were placed into one-liter sterilized plastic bottles along with an individual 100 mL portion of PO4 buffer w/ 0.1% Tween. The bottle was shaken on an orbital shaker for 20 minutes. The carriers were then aseptically removed from the container.

10.5. Enumeration

Surviving challenge organisms were enumerated using appropriate serial dilutions and pour over plating. Serial dilutions were made in sterile H_20 and overpoured with TSA. TSA plates were incubated at 30-35°C for 3 days.

11. Calculations

The Log Reduction Value (LRV) was calculated and reported for each test group using the following equation:

Log Reduction = $\log_{10}N_0 - \log_{10}N_1$

N_0 = average bacterial challenge determined from the positive (untreated) controls, measured in colony forming units (CFU).
N_1 = average number of bacteria surviving UVGI exposure measured in CFU. If $N_1 < 1$, then use $N_1=1$.

12. Results

Recovery Method Validation – 10 minute inoculum dry time

Organism	Inoculum Population (CFU)	Mean Recovery (CFU)	% Recovery	Pass/Fail
E. coli	40	51	128%	Pass
S. aureus	63	54	86%	Pass

Note: initial Recovery Method Validation using 30-45 minute inoculum dry time, as specified in protocol 2020-RES-044, resulted in poor % recovery (E. coli = 28%, S. aureus = 20%). Inoculum dry time was decreased to 10 minutes and validation repeated. After acceptable results, the same 10 minute dry time was used for the UVGI Disinfection Trials.

UVGI Disinfection Trials

Organism	Group	Non-treated Control (CFU)	Log of Control	After Treatment (CFU)	Log treated samples	Log Reduction
E. coli	Group 1 (45 sec)	5.0×10^6	6.7	2	0.3	6.4
	Group 2 (90 sec)	5.0×10^6	6.7	<1	0	6.7
S. aureus	Group 1 (45 sec)	5.3×10^6	6.7	<1	0	6.7
	Group 2 (90 sec)	5.3×10^6	6.7	<1	0	6.7
N/A	Group 4 (neg. control)	N/A	N/A	<1	0	N/A

13. Records

All raw data, documentation, protocols and final reports will be retained for two (2) years in the archives at iuvo BioScience, 7500 West Henrietta Road, Rush, New York 14543. After two (2) years the sponsor will be contacted for final disposition of documentation.

14. Attachments

Attachment I	Pictures of the inoculation sites

Attachment I

Picture of the inoculation site