Study Report: GLP2941

GLP Device Study Based on the ASTM E1153 Surface Time Kill Against S. aureus (MRSA)

UVC Disinfecting Cabinet, Model UVC-18-75-1

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2-6-2022

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BRIEF OVERVIEW OF PRODUCT

The UVC Disinfecting Cabinet, Model UVC-18-75-1 is manufactured by UVC Hygenix Inc dba PureWorks.

The UVC LED Disinfecting Cabinet is designed to decontaminate contents from harmful viruses and bacteria while providing a safe environment for the workplace. By optimally positioning UVC LED modules inside the cabinet to eliminate blind spots, contents are disinfected within one minute. Additional safety features include a digital timer and an interlock cabinet door to ensure the UVC modules cannot be powered on until the cabinet door is securely closed. Controls include On-Off Power, timer control, safety door interlock, and in-use LED indicator. This technology is covered by one or more patent applications, including U.S. Pat. App. S/N 62/706,059.

An image of the cabinet is shown in Figure 1 below.

Figure 1 UVC Disinfecting Cabinet, Model UVC-18-75-1

1 INTRODUCTION

UVC Hygenix Inc DBA PureWorks, the manufacturer of UVC Hygenix Inc subcontracted Blackbriar Regulatory Services, LLC (BRS) to conduct studies supporting the label claim about the device's efficacy against the specified microorganisms.

BRS, in collaboration with a qualified laboratory (Michrochem Laboratory, Texas, USA) prepared a Study Protocol using experimental design and specified test method.

Testing was performed in accordance with Good Laboratory Practice Standards (GLPS) stipulated by US EPA 40 CFR Por1 160 as well as the US EPA Product Performance Test Guidelines outlined in OCSPP 810.2200.

Blackbriar Regulatory Services, LLC (BRS) was acquired by Accorto Regulatory Solutions, LLC, while the studies were still ongoing. Upon completion of the testing, the report was issued by Accorto Regulatory Solutions, LLC (Accorto).

2 PURPOSE

The purpose of this study was to document the antimicrobial resistance and susceptibility of the test device against the test system (microorganism) under the test parameters specified in this protocol. The device's efficacy was evaluated using a test method based on ASTM E1153 Surface Time Kill Test. The test protocol was prepared with the intention to verify specific antimicrobial claims supported by relevant test systems (microorganisms) outlined in the US EPA 40 CFR 160 and in EPA Product Performance Test Guidelines, OCSPP 810.2200, Disinfectants for Use on Environmental Surfaces - Guidance for Efficacy Testing.

3 SCOPE AND APPLICATION

The test device was subjected to a microorganism that had been dried onto the surface of a carrier (representing a hard, nonporous surface) and held for the specified contact time. At the conclusion of the contact time, the recovered microorganism was assayed. Plate recovery and cell culture controls were performed concurrently with the test.

3.1 Test Device

The details for the test Device, UVC Disinfecting Cabinet, are summarized in Table 1 below.

Table 1 Test Device Description

Item	Description
Test Device	UV-C LED Disinfecting Cabinet (or UVC Disinfecting Cabinet)
Model	UVC-18-75-1
Received date	11/09/2021
Form	UV-C Device
Storage Conditions	Ambient room temperature

3.2 Experimental Design Information

The primary information for the experimental design is summarized in Table 2 below.

Details can be found in the Study Report for GLP2941, titled " GLP Device Study Based on the ASTM E1153 Surface Time Kill Against S. aureus (MRSA)

Table 2 Experimental Conditions and Details

Item	Description
Study Title	GLP Device Study Based on the ASTM E1153 Surface Time Kill Against S. *aureus* (MRSA)
Study ID	GLP2941
Protocol ID	P3643
Test Microorganism	Staphylococcus aureus ATCC 33592 (MRSA)
Organic Soil Load	none
lnoculum Volume	0.020 ml
Carrier Type	Sterile Glass Slides (1 x 3 inch)
Number of Test Carriers	Two
Contact Time	l minute

Table 2 Experimental Conditions and Details

Item	Description
Exposure Temperature	Ambient room temperature (22.8° C) and relative humidity (RH)(33.2%)
Neutralization Method	NA
Experimental Start Date/Time	12/20/2021/ 12:18
Experimental End Date/Time	12/22/2021/14:20
Study Completion Date	01/28/2022

3.2.1 Test Procedure

Staphylococcus aureus ATCC 33592, originally received from the American Type Culture Collection (ATCC), Manassas, VA, was used in this study.

Staphylococcus aureus ATCC 25923 (reference), originally received from the American Type Culture Collection (ATCC), Manassas, VA, was also used in this study

The test device was set up and operated per device manual. The interior of the device was not wiped down prior to testing. The device rack was placed on the top level during testing. The test device was placed on a flat surface and powered on when the device switch was flipped to the ON position, as indicated by the green light on the front of the device. Photos of the setup were taken. A device warm-up cycle was not performed prior to testing.

An appropriate number of sterile 1" x 3" glass slides, free from scratches, chips, or cracks, were soaked in 70-95% reagent alcohol to remove oil and film. The carriers were then thoroughly rinsed in tap water, followed by two rinses in deionized water and dried using a lint-free cloth. The dry carriers were placed on a drying rack, covered in aluminum foil and autoclave sterilized on a fast/dry cycle for ≥20 minutes at approximately 121° C. The carriers were placed into a 36±1°C incubator to dry after sterilization. Inside a biosafety cabinet, sterile carriers were aseptically transferred using sterile forceps to individual Petri dishes.

The test culture was transferred from the monthly working stock using a sterile inoculating loop into 10mL of sterile tryptic soy broth (TSB) and incubated at 36 ± 1° C for 23 hours and 6 minutes. The culture was transferred once using a sterile inoculating loop to transfer 0.10 mL of the culture into 10 mL of sterile TSB. The final test culture was incubated at 36 ± 1° C for 49 hours and 14 minutes. The test culture was harvested and vortex mixed, then allowed to dwell for 15 minutes and 12 seconds. No further manipulation of the test culture was performed. The inoculum was plated to determine the starting concentration of microorganisms in CFU/mL. The concentration was 9.50 x 108 CFU/mL.

A total of six carriers were inoculated with 0.020 mL of the test culture in a biological safety

cabinet. The inoculum was spread on approximately 1 in2 of each carrier, ensuring the inoculum did not touch the edge. Each carrier was covered immediately after inoculation. The carriers were then placed in an environmental chamber to dry for 48 minutes and 1 second at 35.8 − 35.9°C in a relative humidity of 43 − 44%.

Two dried, inoculated carriers were placed uncovered on the test device rack, and the device door was shut and secured. The timer on the device was set to 1 minute, and the blue button pressed to begin treatment at an ambient exposure temperature of 22.8° C in a relative humidity of 33.2%. The red indicator light was on for the duration of 1 minute. At the conclusion of the contact time, once the device had been turned off, each carrier was removed from the device and immediately harvested into conicals containing 20 mL of sterile PBS, supplemented with 0.1% Tween-80 (PBST). After harvesting, the carriers were then vortex mixed for 1 minute.

The following controls were used during the experiment:

- Harvesting of Control Carriers
- Enumeration of Test and Control Carriers
- Test Substance Neutralization Control
- Carrier Sterility Control
- Media Sterility Control
- Test Microorganism Purity Control

3.2.1.1 Antimicrobial Resistance and Susceptibility Assay

The test and reference strains were transferred from the monthly working stock using a sterile inoculating loop onto sterile tryptic soy agar. The cultures were incubated at 35.7° C for 24 hours and 14 minutes. A selection of 5 isolated colonies was harvested from each test and reference plate, respectively, and transferred into 5 ml of sterile TSB and incubated at 35.7° C for 4 hours and 23 minutes. Once removed from incubation, the cultures were diluted 1:5 in sterile phosphate-buffered saline to achieve approximately 1.5 x 108 CFU/ml. The diluted cultures were compared to a 0.5 McFarland standard and used within 15 minutes. The inoculum was plated and incubated alongside the assay materials to confirm concentration. The test microorganism concentration was 1.64 x 108 CFU/ml, while the reference microorganism concentration was 1.40 x 108 CFU/ml. To perform the assay, a sterile swab was dipped into the prepared culture and pressed against the wall of the tube while rotating several times above the fluid level. The inoculated swab was then rubbed over the entire surface of a solidified Mueller Hinton Agar (MHA) plate. The plate was then rotated 60° C and the entire plate was swabbed again, using the same swab. This process was performed two more times, and after the final swab the rim of the plate was swabbed to ensure microorganism coverage to the edge of the agar. This process was followed for both the test and reference microorganisms. Separate plates were

swabbed in the same manner to confirm the confluent growth of the microorganisms. After swabbing, the plates were left with the lids ajar for 3 minutes and 30 seconds prior to placing the antimicrobial disk. The disk, Cefoxitin at 30 μg concentration, was lightly pressed into the agar and the plates were inverted. All plates were incubated at 35.7 − 36.0° C for 17 hours and 34 minutes. After incubation, the plates were observed for zones of inhibition (ZOI), confluent growth and to ensure the appropriate inoculum concentration was achieved. The ZOI for the test and reference strains were measured using calipers. The reference strain had a ZOI of 22.4mm at a concentration of 1.40 x 108 CFU/mL. The test strain had a ZOI of 6.5mm at a concentration of 1.64 x 108 CFU/mL.

All test materials were incubated for 47 hours and 57 minutes at 35.9° C − 36.0° C.

Data obtained from the final reading are documented in the Results section of this report.

3.3 Acceptance Criteria

The experimental success (controls) criteria follow::

- The test microorganism must demonstrate a concentration of at least 1.0×10^5 CFU/Carrier.
- All media sterility controls demonstrate no growth.
- The carrier sterility control demonstrates no growth.
- The test microorganism purity control plate demonstrates the presence of the target
- microorganism and the absence of contaminant microorganisms.
- Inoculum for antimicrobial susceptibility testing must be within 0.5 logs of 1.5 x 108 CFU/ml.
- Microorganisms' susceptibility guidelines are as follows:
  - The test microorganism must demonstrate a zone of inhibition of ≤21 mm against Cefoxitin (30 μg) to be considered Oxacillin resistant.
  - The reference microorganisms must demonstrate a zone of inhibition ≥22 mm against Cefoxitin (30 μg) to be considered Oxacillin susceptible.

4 STUDY RESULTS

Study results are summarized in Table 3 and Table 4 below.

Table 3 Study Results Summary

Test Microorganism	Carrier Designation	Replicate	CFU/Carrier	Average CFU/Carrier	Percent Reduction Compared to Control	Log10 Reduction Compared to Control
S. *aureus* ATCC 33592	Control	1	1.46E+07	1.57E+07	N/A	
		2	1.68E+07			
	Test	1	2.26E+03	3.62E+03	99.98%	3.64
		2	4.98E+03			

Table 4 Antimicrobial Resistance and Susceptibility Assay Results

Microorganism	Dilution	Inoculum CFU/ml	Zone of Inhibition (mm)	Result Valid?
S. aureus ATCC 33592 (Test)	1 : 5	1.64E+08	6.5	Yes
S. aureus ATCC 25923 (Ref.)	1 : 5	1.40E+08	22.4	Yes

Detailed data for each test can be found in Study Report GLP2941.

5 STUDY CONCLUSIONS

The purpose of the study was to determine the efficacy of UV-C LED Disinfecting Cabinet (model: UVC-18-75-1), against *Staphylococcus aureus* ATCC 33592 (MRSA), with no organic soil load, at a contact time of 1 minute at ambient room temperature (22.8° C) and relative humidity (33.2%).

The evaluated test device, UV-C LED Disinfecting Cabinet (model: UVC-18-75-1), demonstrated an average 3.64 $\log_{10}$ reduction as compared to the corresponding time zero controls.

No microbial contamination of any media or test culture was observed during the course of the study.

The test microorganism demonstrated acceptable resistance to the antibiotic disk (Cefoxitin 30 μg) and the reference microorganism demonstrated acceptable susceptibility to the antibiotic disk (Cefoxitin 30 μg).

This study was carried out in compliance with the approved protocol. All experimental controls met the established acceptance criteria unless otherwise noted in the Protocol Changes in the report.

There were no circumstances that may have affected the quality or the integrity of the data.

6 REFERENCES

- Study Protocol P3643, Titled" GLP Device Study Based on the ASTM E1153 Surface Time Kill Against *S. aureus* (MRSA)", by Microchem Laboratory
- Study Report GLP2941 Titled" GLP Device Study Based on the ASTM E1153 Surface Time Kill Against *S. aureus* (MRSA)", by Microchem Laboratory, completed 01/28/2022