Study Report: GLP2943

Custom Virucidal Efficacy of an Ultraviolet Light Disinfection Device: Influenza A (H1N1) virus, NPR/8/34 strain, ATTC VR-1469

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

PUREWORKS

PROVEN PURIFYING POWER

Accorto Regulatory Solutions, LLC
11533 Busy Street Unit #215
North Chesterfield, VA 23236
2-3-2022

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

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 US Pat. App. S/N 62/706,059.

An image of the cabinet is shown in Figure 1 UVC Disinfecting Cabinet, Model UVC-18-75-1Figure 1 below.

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

1 INTRODUCTION

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

BRS, in collaboration with a qualified laboratory (Michrochem Laboratory, Texas, USA) prepared a Study Protocol using experimental design and modified ASTM E1053 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 virucidal efficacy of the test device against the test system (microorganism) under the test parameters specified in this protocol. The test protocol was prepared with the intention to verify specific antimicrobial claims supported by relevant test systems (microorganisms) outlined in the 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 film of virus that had been dried onto the surface of a glass carrier (representing a hard, nonporous surface) and held for the specified contact time. At the conclusion of the contact time, the recovered virus was assayed for infectivity. Plate recovery, virus inoculum titer, 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 GLP2943, titled "Custom Virucidal Efficacy of an Ultraviolet Light Disinfection Device."

Table 2 Experimental Conditions and Details

Item	Description
Study Title	Custom Virucidal Efficacy of an Ultraviolet Light Disinfection Device
Study ID	GLP2943
Protocol ID	P3642
Test Microorganism	Influenza A (HlNl) virus, NPR/8/34 strain, ATCC VR-1469
Host Cell	MDCK cells (NBL-2) (ATCC CCL-34)
Organic Soil Load	5% Fetal Bovine Serum
lnoculum Volume	0.200 ml
Carrier Type	Sterile glass Petri dish (150 mm x 20 mm)
Number of Test Carriers	Two
Contact Time	1 minute

Table 2 Experimental Conditions and Details

Item	Description
Exposure Temperature	Ambient room temperature (18.9-19.3 °C} and 49.4-50.1% relative humidity (R.H.)
Neutralization Method	Eagle's Minimum Essential Medium (EMEM) supplemented with 2% FBS test media (2.0ml)
Experimental Start Date/Time	01/04/2022 /13:49
Experimental End Date/Time	01/11/2022/ 08:49
Study Completion Date	01/19/2022

3.2.1 Test Procedure

Influenza A (HlNl) virus, A/PR/8/34 strain, ATCC VR-1469, originally received from the American Type Culture Collection (ATCC), Manassas, VA, was used in this study. The lot number used in testing was Hl Nl _07NOV202 l.

The test virus was propagated internally by inoculating the virus into cell culture flasks containing the appropriate host cell line and incubating at the appropriate conditions. Once the cell culture flasks displayed approximately 75-100% cytopathic effect (as determined by a microscopic evaluation), the flasks were subjected to freeze-thaw cycles to release virus from infected cells. The contents of the cell culture flasks were collected and centrifuged in order to remove the cell debris. The test virus was then aliquoted and stored at -70 °c.

On the day of testing, the appropriate number of virus stock suspension vials were removed from cryostorage and thawed for use in the assay. The test virus contained a 2% fetal bovine serum (FBS) organic soil load. The test virus was adjusted to contain 5% FBS organic soil load by adding 0.030 ml of FBS to 0.950 ml of test virus.

MOCK cells (NBL-2) (ATCC CCL-34), originally received from the ATCC, were utilized in the assay. The cells were subcultured and seeded into 24-well cell culture plates. The plates were incubated at 36±2 °C in a humidified atmosphere of 6± 1 % CO_2 until they reached the desired confluence required for testing. On the day of use, the cells were microscopically examined to verify the appropriate confluency and health of the cells. Cell culture passage documentation, including cell culture source, passage number, seeding densities,etc., was retained.

The test medium utilized in the assay was Eagle's Minimum Essential Medium (EMEM) supplemented with 2 µg/ml TPCK trypsin and bovine serum albumin (BSA) 40 mM HEPES buffer,125 µM non-essential amino acids, 1 mM sodium pyruvate, plus antibiotics (antibiotic-antimycotic solution (100 units/ml penicillin G, 100 µg/ml streptomycin, and 0.25 µg/ml amphotericin B)]. Concentrations based on the preparation of 1 L of Eagle's Minimal Essential Medium.

An appropriate number of sterile glass Petri dishes free from scratches, chips, or cracks were soaked in 70-95% reagent alcohol for 30 minutes to remove oil and film. The carriers were then thoroughly rinsed using two separate DI water rinses and dried. The dry carriers were placed in single layers on autoclavable trays lined with absorbent towels and autoclave sterilized on a fast/dry cycle for 20 minutes at approximately 121°C.

The test virus was vortexed thoroughly and a 0.200 ml aliquot of virus was placed on the inside bottom surface of four 150 mm x 20 mm sterile glass Petri dishes which served as the test carriers and plate recovery control. The inoculum was spread over the entire area of the carriers using a sterile bent pipette tip without touching the sides of the Petri dish. The virus films were dried in an environmental chamber for 13 minutes at 20.0 °C in a relative humidity of 29%.

The test device was set per the instructions/device manual. Prior to testing the device, inside surfaces, including the device rack, were wiped with a microfiber cloth containing reagent alcohol. The device rack was placed on 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 (see page 16 for images). The device warmup cycle was performed prior to testing.

One dried virus film carrier was 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 the requested exposure temperature of 20.9-21.1 °C in a relative humidity of 26%. The red indicator light was on for the duration of 1 minute. At the end of 1 minute, the green light illuminated. At the conclusion of the contact time, once the device had been turned off, the carrier was removed from the device, covered with the lid, transferred to a biological safety cabinet, and a 2.0 ml aliquot of test media was added to the carrier. The carrier was gently rotated to ensure complete coverage of the solution over the entirety of the surface. Using sterile cell scrapers, the carrier was scraped to re-suspend the viral film, and the suspension was transferred to sterile vessels. Serial 10-fold dilutions using 0.1 ml of appropriate recovery fluid (test media) and 0.9 ml of test assay media were prepared to the appropriate dilution. The same process was repeated for a second dried virus film carrier.

The following controls were used during the experiment:

- Plate recovery controls (2)
- Cytotoxicity controls
- Test Substance Neutralization controls
- Cell culture control
- Virus inoculum titer control

3.2.1.1 Infectivity Assay

A 0.1 ml aliquot of all test and control dilutions was inoculated into the host cell cultures (which contained test medium) in quadruplicate. To facilitate virus-host cell adsorption, an adsorption step was performed by inoculating the dilutions into the host cell cultures, which did not contain test medium. The assay plates were incubated at 36±2°C in a humidified atmosphere

of 6± 1% CO2 for30 minutes. Following adsorption, each well received an approximate 1.0 ml aliquot of test medium via pipette delivery, and the assay plates were incubated at 36±2 °C in a humidified atmosphere of 6± 1% CO2 for ~ 7 days. The assay plates were examined microscopically periodically throughout the incubation period with any changes to the Data obtained from the final reading are documented in the Results section of this report.

3.3 Acceptance Criteria

The following measures are met to ensure the acceptability of virucidal efficacy data:

- The virus titer control demonstrates obvious and/or typical cytopathic effects on the monolayers unless a detection method other than the cytopathic effect is used.
- A minimum of 4.80 Log_{10} infective units/control carrier is recovered from each plate recovery control film(s).
- Quantification of the test and control parameters is conducted at a minimum of four determinations per dilution.
- The cell controls are negative for infectivity and demonstrate typical cell morphology.

The product performance criteria are as follows:

- The log and percent reduction of the test virus following exposure to the test device is calculated; however, there is no minimum reduction level to qualify as "passing" or an "efficacious" product.
- For liquid/spray/towelette products, the US EPA performance criteria for disinfection are as follows:
  - In the presence or absence of cytotoxicity, the product should demonstrate a 3.00 log_{10} reduction in viral titer on each surface.

4 STUDY RESULTS

Study results are summarized in Table 3.

Table 3 Study Results Summary

Description	**Assay Results**		
	Test Device Name: UV-C LED Disinfecting Cabinet Test Device Model Number: UVC-18-75-1		**Plate Recovery Control**
	Test Replicate 1	Test Replicate 2	6.30 Log_{10} Avg $TCID_{50}$ Carrier
Log_{10} $TCID_{50}$/ 0.1 ml	≤0.75 Log_{10}	≤0.50 Log_{10}	
Log_{10} $TCID_{50}$/ Carrier	≤1.05 Log_{10}	≤0.80 Log_{10}	
Avg. Log_{10} $TCID_{50}$/ Carrier	≤0.94 Log_{10}		
Avg. Log10 Reduction / Carrier	≤5.36 Log_{10}		

Detailed data for each test can be found in Study Report GLP2943.

5 STUDY CONCLUSIONS

The Virucidal Efficacy for the test Device was confirmed, and all acceptance criteria set in the protocol was met for UV-C LED Disinfecting Cabinet (model: UVC-18-75-1), against Influenza A (HlNl) virus, A/PR/8/34 strain, ATCC VR- 1469, supplemented with a 5% fetal bovine serum (FBS) soil load, at a contact time of l minute and exposure temperature of room temperature (20.9-21.1 °C and 26% relative humidity (R.H.)).

The Plate Recovery Control demonstrated an average viral titer of 6.30 Log_{10} $TCID_{50}$ per carrier, thereby satisfying US EPA study acceptance criteria of a minimum of 4.80 Log_{10} infective units per control carrier.

The evaluated test device, UV-C LED Disinfecting Cabinet (model: UVC-18-75-1), demonstrated an average ≥5.36 Log_{10} reduction in viral titer as compared to the corresponding plate recovery control.

No microbial contamination of any host cell cultures was observed during the course of the study.

This study was carried out in compliance with the approved protocol. All experimental controls met the established acceptance criteria.

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

6 REFERENCES

- Study Protocol P3642, Titled Custom Virucidal Efficacy of an Ultraviolet Light Disinfection Device", by Microchem Laboratory
- Study Report GLP2943 Titled Custom Virucidal Efficacy of an Ultraviolet Light Disinfection Device", by Microchem Laboratory, completed 01/19/2022