**Clostridium difficile** Spore Inactivation Study Using Ultraviolet-C Energy

May 2012

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**OBJECTIVE:** To determine inactivation rates for *Clostridium difficile* spores by irradiating inoculated coupons with ultraviolet-c (UV-C) energy generated and measured by the R-D™ Rapid Disinfector™ UV-C System (the System) and its remote “challenge devices”.

**METHOD:** This study was carried out in a controlled laboratory environment setting. *Clostridium difficile* spores were placed in a laboratory room, in direct line of sight of the UV-C emitter device (the Emitter). This study only used definitive, preselected UV-C dose settings and was not based on treatment time or the distance the coupons were located from the Emitter. The dose delivered was measured by the System's remote UV-C sensor “challenge devices”. Independent tests were performed using six (6) different UV-C doses with three (3) coupons for each dose. After each controlled UV-C dose was delivered to each set of coupons the remaining viable *Clostridium difficile* spore colony counts were determined to compute the reduction from positive control coupons that were not irradiated.

**RESULTS:** In this test the effectiveness of UV-C radiation in reducing the spore count of *Clostridium difficile* ranged between 3.4 - 4.4 log10 after delivering a measured dose ranging from 45,903 to 159,693 µW-sec/cm².

**CONCLUSION:** The R-D Rapid Disinfector UV-C System was highly effective in reducing *Clostridium difficile* spores on contaminated surfaces.

**OVERVIEW:**

Surface disinfection of noncritical surfaces and equipment is normally performed by manually applying a liquid disinfectant to the surface with a cloth, wipe, or mop. Recent studies have identified substantial opportunities in hospitals to improve the cleaning of frequently touched objects in the patient’s immediate environment.1-3 For example, of 20,646 standardized environmental surfaces (14 types of objects), only 9,910 (48%) were cleaned at terminal room cleaning.3 Epidemiological studies have shown that patients hospitalized in rooms previously occupied by individuals infected or colonized with methicillin-resistant *Staphylococcus aureus* (MRSA),4 vancomycin-resistant *Enterococcus* (VRE),5 or *Clostridium difficile*6 are at significant risk of acquiring these organisms from contaminated environmental surfaces. These data have inspired the development of room decontamination devices that avoid the problems associated with manual disinfection.3

Devices using UV-C light (wavelength=254nm) have also been proposed for room decontamination. The device used herein utilizes remote wireless UV-C “challenge device” sensors, each with a dynamic range 0-250 µW-sec/cm², which definitively determines when all targeted treatment areas have received a predetermined dose of UV-C energy necessary to provide the desired reduction of the specific microorganism(s). Since fast room treatment time is desired the System is designed to allow the operator to pause, reposition, and resume a treatment. This feature allows the operator the ability to eliminate shadowed areas thereby reducing treatment time and increasing efficacy. Objects in closer proximity to the Emitter than the sensors (high touch surface areas including bed rails, tray tables, and other medical devices located around the patient bed) receive significantly greater dosages of UV-C energy because of the inverse square law of light energy. The System is fully automated, has a self contained computer, is activated by a wireless PDA hand-held remote control, and does not require closing off of the room HVAC system. It measures direct UV-C light from the Emitter and is pre-programmed with the definitive UV-C radiation dose required.
to kill Clostridium difficile spores (for example, and other targeted microorganisms). The System does not rely on time based or distance based solutions for the delivery of UV-C energy, neither of which can definitively deliver a prescribed dose to a targeted area. After each of the remote wireless sensors receive the prescribed dose for decontamination, the System automatically captures data that reports System ID, job ID, date and time of decontamination, operator, room location, dose received, elapsed time and final completion status. The purpose of this report is to determine the level of inactivation of Clostridium difficile spores for various amounts of UV-C energy delivered and measured by the R-D Rapid Disinfector System.

LABORATORY TEST RESEARCH:

A single UV-C emitting device was investigated (R-D Rapid Disinfector, Steriliz, LLC). This device delivers a preset dose of UV-C radiation to the areas to be treated. The dose used is based upon the inactivation dose needed to reduce the targeted pathogen to some preselected level (3 Log_{10}, for example). At the time of this study there was no known published UV-C inactivation dosage data for Clostridium difficile. As such, the sponsor arbitrarily established six (6) discrete dosages ranging from 45,903 to 159,693 µW-sec/cm² to provide a range of inactivation results.

Three (3) Formica coupons inoculated with Clostridium difficile were placed in the lab adjacent to the System's “challenge device” UV-C sensors and once the room was vacated they were irradiated with the first dose of UV-C energy – 45,903µW-sec/cm². After irradiation completed the coupons were removed for processing. This protocol was repeated for the remaining five (5) sets of coupons and their respective doses. Positive control was provided by three (3) coupons that were not irradiated.

RESULTS:

In this test the effectiveness of UV-C radiation in reducing the counts of C. difficile spores was >99.9%. The total CFU log_{10} reduction is shown in Table 1 Microbial recoveries/Log reduction data; 5-7-2012. The UV-C dose delivered was measured by the System's remote cosign corrected UV sensors and a portable radiometer that were placed in the lab test room. After treatment, there was a significant reduction in total CFUs as indicated in Table 1 and Graph1 Microorganism log reduction vs. Dosage.

DISCUSSION:

UV irradiation has been used for the control of pathogenic microorganisms in a variety of applications, such as control of legionellosis, as well as disinfection of air, surfaces, and instruments. At certain wavelengths, UV light will break the molecular bonds in DNA, thereby destroying the organism. UV-C has a characteristic wavelength of 200–270 nm, which lies in the germicidally active portion of the electromagnetic spectrum of 200–320 nm. The efficacy of UV irradiation is a function of many different location and operational factors, such as intensity, exposure time, lamp placement, and air movement patterns. These studies showed that this technology is an effective, environmentally friendly method to disinfect surfaces.

The system evaluated is unique in that it uses remote “challenge device” UV-C sensors to measure the definitive UV-C intensity and dosage delivered to each point of interest. UV-C irradiation remains active until the programmed lethal dose of energy for the specified microorganisms has been delivered to the actual targeted areas. The ability of the device to deliver lethal doses of UV-C light energy to epidemiologically important microorganisms on surfaces was evaluated. It was shown that the quantities of these organisms were significantly reduced (by >3–4 log_{10}) under contamination levels that exceed the levels normally found in healthcare facilities. In fact, studies have shown that, although the frequency of contamination by these pathogens (e.g., C. difficile) is high (10% to more than 50%), the microbial load is generally low (less than 10 to 100 CFUs per plate or sample).

In these experiments, surfaces were not pre-cleaned prior to treatment with UV-C. However, because the presence of dirt and debris can decrease the effectiveness of UV-C disinfection, areas to be irradiated with UV-C light should be manually pre-cleaned with approved disinfecting agents. Wiping all surfaces and
objects with an Environmental Protection Agency–registered disinfectant, in accordance with the product instructions should take place prior to UV-C irradiation.

The advantages of the R-D™ Rapid Disinfector™ System include:
- The use of remote wireless precision calibrated electronic incident light sensors, “challenge devices”, to definitively measure actual UV-C light delivered to targeted treatment areas (compared to other systems which only guesstimate delivered light);
- The ability to pause, reposition, and resume a disinfection job to maximize efficiency thereby reducing treatment time and eliminating shadowed areas in the environment;
- The system is portable and can be used throughout a facility to disinfect more areas, faster;
- Space may be occupied immediately after treatment; average cycle time per room ~10-20 minutes, much faster than other UV-C products;
- Online “real time” data collection, reporting and analysis;
- HVAC system do not need to be sealed to prevent UV light from escaping;
- Time proven steady-state UV-C technology - no pulsating light or sounds - R-D is a silent process;
- The system comes equipped with a simple to use door safety system;
- There are no consumable products.

The disadvantages include the following:
- More scientific studies are needed to determine whether a significant reduction in pathogens in the environment will result in a reduction in infection rates;
- Decontamination protocol which includes UV-C treatment needs to be adhered to;
- Area to be treated must be free of humans, plants and animals;
- UV-C does not penetrate fabrics.

SUMMARY:

UV-C technology offers an option for room decontamination, especially in healthcare facilities. *C. difficile* spores are epidemiologically important pathogens that have an environmental mode of transmission. Because contamination of environmental surfaces is common even after manual surface disinfection (which is proven to be not very effective), and because contamination of healthcare worker hands can transfer these pathogens to patients, resulting in substantial numbers of infections, this technology (and other effective room decontamination technologies) should be considered for use in selected patient rooms and care areas to augment current surface disinfection practices..

REFERENCES:

Final Report: Revision 1

Evaluation of Steriliz® R-D Rapid Disinfector Against Surface Contamination of Clostridium difficile on Formica® Surfaces

Written & Approved by:

[Signature]
Patrick Polito
Microbiologist I

20.3.12
Study Completion Date
Test Facility: MMDG: Life Science Laboratories  
7500 West Henrietta Rd.  
Rush, NY 14543

Purpose of the Study: To evaluate the effectiveness of the R-D Rapid Disinfector against Clostridium difficile spore, hereby referred to only as Clostridium difficile, inoculated on the surface of Formica® coupons.

Test Equipment: All information regarding the R-D Rapid Disinfector was be provided by the sponsor

Control Material: Formica® coupons inoculated with Clostridium difficile and not exposed to the R-D Rapid Disinfector served as inoculation controls. Uninoculated sterile coupons served as negative controls

Materials: Challenge Organism

Clostridium difficile ATCC # 700792

Sterile Media

Trypticase Soy Agar (TSA)  
Synthetic Sporulation Medium 10  
Reinforced Medium for Clostridia MLT  
Phosphate buffer with 0.1% TWEEN 80  
TS Saline  
Water Blanks

Additional Equipment

Spectrophotometer  
Colony Counter  
Centrifuge  
Mechanical Shaker  
Incubators: 20-25°C, 30-35°C, 45-50°C (agar medium equilibration)  
Pipette aid  
Vortex  
Sterile Formica® Coupons  
General Microbiological glassware

Responsibilities: The test sponsor was responsible for set up and operation of the R-D Rapid Disinfector device, documentation of dosages, and recording any data generated from this device. The testing laboratory provided the testing environment, supplies and challenge organism as well as the preparation of the test protocol and report, documentation of laboratory testing conditions and physical manipulation of the challenge organism post exposure to the disinfecter device.
Procedures:

**Spore Suspension Preparation:**

The *Clostridium* spp. was subcultured onto fresh Reinforced Medium for *Clostridia* MLT. The Reinforced Medium for *Clostridia* MLT was incubated at 30-35°C for at least 12 hours.

The challenge organisms was transferred into sterile 50 ml centrifuge tubes. The samples were centrifuged at 4,000 RPM for eight (8) minutes, the supernatant decanted and the pellet re-suspended in 20 ml of Synthetic Sporulation Medium 10. The organisms in sporulation medium were incubated at 30-35°C for approximately two (2) weeks.

Following incubation, the samples were vortexed for at least 30 seconds then serially diluted in sterile water. Non-heat shock and heat shock (heat shock at 80 °C for 15 minutes) population determinations were performed on the sample to determine if at least 70% sporulation was obtained.

Duplicate 1.0 ml aliquots of the non-heat shocked and heat shocked dilutions were transferred to sterile petri plates. All plates were overpoured with sterile Trypticase Soy Agar (TSA) maintained at 45-50°C. All plates were inverted and incubated at 30-35°C for 24-48 hours. Colonies were counted using a colony counter and counts recorded.

Upon obtaining at least 70% sporulation of organisms, the spore suspension was removed from incubation and stored at 2-8°C (refrigeration).

**Preparation of Standardized Inoculum**

The challenge organisms were spectrophotometrically adjusted in sterile TS Saline to a concentration of greater than 10^7 colony forming units (cfu) /ml. Suspensions will be post diluted prior to testing.

**Challenge Procedure**

Three (3) sterile test coupons per dose and three (3) sterile control coupons for positive controls were individually inoculated with 1.0 x 10^5 to 1.0 x 10^6 of the challenge organism. This equated to a total of eighteen (18) coupons for the antimicrobial challenge and three (3) coupons for population (positive) controls. Three (3) un-inoculated coupons served as negative controls.

The coupons were arranged evenly spaced, three per dosage on a horizontal surface Parallel to the disinfectant and at a distance of approximately eleven (11) feet.

Following dosing, three (3) of the test coupons for each dose were dropped into 100 ml of sterile Phosphate buffer with 0.1% TWEEN 80, mechanically agitated for a minimum of 20 minutes, then serial diluted with nine (9) ml sterile water blanks and appropriate dilutions were plated in duplicate with TSA.
Dosing

The test sponsor dosed each set of coupons separately with the following sequential doses of UV light in microwatt seconds per sqcm. Following each dose, the unit was shut down, a set of coupons was retrieved for testing, a new set of coupons was placed appropriately, and dosing continued again once personnel were longer present in the room.

- 45,903
- 68,651
- 90,279
- 113,450
- 135,792
- 159,693

Control and Microbial Recovery Procedure

At the start of the testing, three (3) positive control coupons were dropped into 100 ml of sterile Phosphate buffer with 0.1% TWEEN 80, mechanically agitated for a minimum of 20 minutes, then serial diluted with nine (9) ml sterile water blanks and appropriate dilutions were plated in duplicate with TSA.

Three (3) un-inoculated negative control coupons were dropped into 100 ml of sterile Phosphate buffer with 0.1% TWEEN 80, mechanically agitated for a minimum of 20 minutes, and one (1) ml from each was plated in duplicate with TSA.

Following each dosage, three (3) of the coupons were dropped into 100 ml of sterile Phosphate buffer with 0.1% TWEEN 80, mechanically agitated for a minimum of 20 minutes, then serial diluted with nine (9) ml sterile water blanks and appropriate dilutions were plated in duplicate with TSA.

All plates were incubated at 30-35°C for a minimum of 3-5 days, the plates were counted and the counts recorded. The log of the population of the organisms on control coupons was compared the log of organisms remaining of the test coupons.

Results:

The results of the physical microbial recoveries from the Positive Controls (PC) and the coupons inoculated and dosed are presented in table I and graphically represented in graph I. The Steriliz® R-O Rapid Disinfector demonstrated the ability to reduce a population of Clostridium difficile inoculated on Formica by ≥4.4 Log units at the highest dosage tested in this study.
### Table I: Microbial recoveries/Log reduction data; 5-7-2012

<table>
<thead>
<tr>
<th>Coupon</th>
<th>Dosage</th>
<th>Dilution</th>
<th>counts (cfu)</th>
<th>average x -1 df (N0)</th>
<th>LogN0</th>
<th>Avg Log N0</th>
<th>Log reduction vs control population</th>
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</table>
Graph I:

**Microorganism log reduction vs. Dosage**

![Graph showing log reduction vs. dosage](image)

**Conclusions:** All additional conclusions are to be drawn by the sponsor.

**References:**

STS M-047: Transfer of Organisms

STS M-060: Challenge Microorganism Preparation, Harvesting and Spectrophotometric Determination

**Records:** All raw data, documentation, protocols, and final reports generated by this study will be retained in the archives for a minimum of two (2) years at MMDG: Life Science Laboratories, 7500 West Henrietta Rd., Rush, NY 14543. Following two (2) years of archiving, the test sponsor will be contacted to determine further disposition as described in MMDG WI SRP-006.