UV ANGEL LAB TEST: INACTIVATION OF BACTERIAL PATHOGENS: CLOSTRIDIUM DIFFICILE SPORES

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Introduction

UV Angel is an automated anti-microbial device that effectively disinfects targeted surfaces, areas highly trafficked with human interaction, shared public surfaces (e.g. computer technology, medical devices, kiosks, atm, point of sale devices, communication devices, etc…) by way of a low-intensity UV-C bulb and software. The device emits low intensity Ultra Violet C light that has the capability to kill bacterial pathogens present on surfaces. The purpose of the current study was to assess the ability of UV Angel to kill Clostridium difficile spores inoculated onto keys on either an external keyboard or a notebook computer at various exposure times up to 20 minutes. In addition, the cumulative effect of a 7.5 minute exposure followed by no exposure for 5 minutes, followed by a final 7.5 minute exposure on the viability of C. difficile spores was evaluated using the external keyboard only.

Materials & Methods

Device: A UV Angel device, external keyboard, notebook, stand, and appropriate software were supplied by UV Angel.

Organisms: A stock solution of Clostridium difficile spores (ATCC 43255; MMX 4821) was previously prepared anaerobically in sterile saline and stored at 4°C.

Protocol*: Quantitation of inoculum:* The spore suspension used for each assay was quantitated by serial dilution plating onto Brucella Agar (BBL; Lot No. 3182110) supplemented with 5 mg/mL Hemin (Sigma, Lot No. 099K1183), 1 mg/mL Vitamin K (Sigma, Lot No. 108K1088), sterile laked sheep blood (Cleveland Scientific, Lot No. 222832), and a 0.1% taurocholate solution (Sigma, Lot No. 081M0058V) to enhance spore germination.

Sterilization of keys: Keys were removed from the keyboard/notebook, immersed in Spore- Klenz (Steris; Lot No. 676783) for 30 min, rinsed in triplicate with sterile water, dipped in 100% ethanol (Sigma; Lot No. SHBF3888V) for 20 min, and were dried in sterile petri dishes in a BioSafety cabinet. Control studies demonstrated that Spore-Klenz was effective in killing C. difficile spores that had been inoculated onto keys (data not shown).

Inoculation and UV Angel treatment of keys: Ten µL of an inoculum was applied to a total of 21 keys, spreading a thin layer over the surface with a sterile pipet tip. As the keys were drying, the "bed" of the keyboard (empty positions where keys were placed) was exposed to UV Angel for 10 min to decontaminate the area. Three keys served as a "Zero" time point for the assay. Three different keys were then placed back into the top "letter" row on the keyboard and exposed to the UV Angel light source for 2 min. These keys were removed for processing (see below), and the process was repeated with three keys each for 5 min and 10 min exposures. For each time point, 3 keys remained in a covered sterile petri dish exposed to ambient light only approximately 3 feet away from UV Angel.

Enumeration of bacteria from the keys: Fifteen µl of a spore preparation of C. difficile was applied to a total of 27 keys, spreading a thin layer over the surface with a sterile pipet tip. As the keys were drying, the "bed" of the keyboard (empty positions where keys were placed) was exposed to UV Angel for 10 min to decontaminate the area. Three keys served as a "Zero" time point for the assay. Three different keys were then placed back into the top "letter" row on the keyboard and exposed to the UV Angel light source for 5 min. These keys were removed for processing (see below), and the process was repeated with three keys each for 10 min, 15 min, and 20 min exposures. For each time point, 3 additional keys remained in a covered sterile petri dish exposed to ambient light only approximately 3 feet away from UV Angel. These served as the control keys in order to evaluate C. difficile spore survival over time in the absence of UV Angel exposure.

Enumeration of spores from the keys: Each key was placed into a separate sterile 50 mL conical tube (Corning; Cat No. 430290) containing 10 mL of sterile saline, rotated in an incubator shaker at 190 rpm/37°C for 10 min, and vortexed vigorously for 30 sec to remove the organisms. Serial 10-fold dilutions were made with sterile saline. The dilutions were plated onto supplemented Brucella Agar (BBL; Lot No. 3182110) containing 5 mg/mL hemin (Sigma; Lot No. 099K1183), 1 mg/mL vitamin K (Sigma; Lot No. 108K1088), sterile laked sheep blood (Cleveland Scientific; Lot No. 222832) and 0.1% taurocholate (Sigma, Lot No. 081M0058V) to enhance spore germination. The supplemented Brucella agar plates were then incubated anaerobically at 35°C for 46-48 hr. After incubation, the colonies were counted and calculations were conducted to determine the time-kill kinetic for each isolate tested.

Figure 1. Time-kill Kinetic of *Clostridium difficile* ATCC 43255 Spores on Notebook Keys

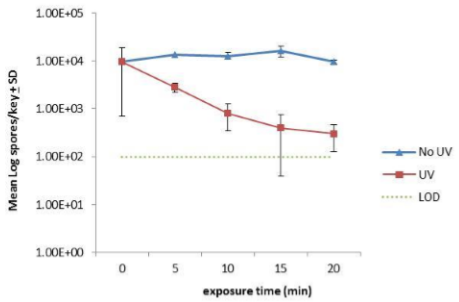


Figure 2. Time-kill Kinetic of *Clostridium difficile* ATCC 43255 Spores on External Keyboard Keys

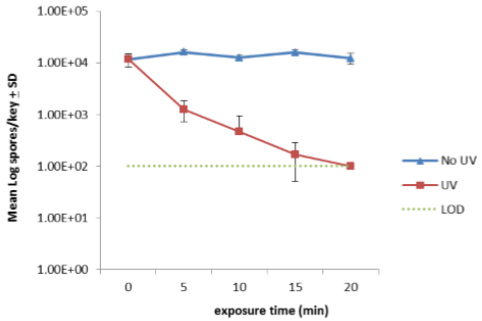
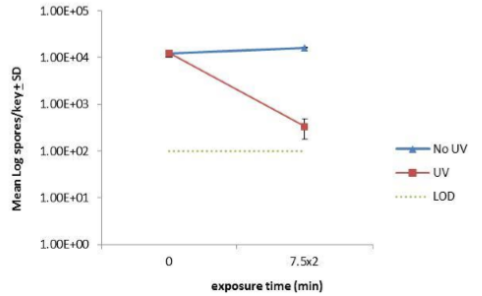


Figure 3. Time-kill of *Clostridium difficile* ATCC 43255 Spores on External Keyboard Keys After Two 7.5 Minute Exposures with a 5 Minute Rest Between Exposures



Results & Discussion

The inoculum was applied with organisms resuspended in sterile saline, followed by drying in ambient air. With the exception of the presence of saline, drying of the inoculum would mimic contamination of keys by human contact. Keys that were exposed to ambient light would provide data for survival of individual organisms under normal conditions, whereas treatment with UV Angel would reveal the ability of the instrument to kill organisms. Methodology was developed to sterilize keys, inoculate known amounts of pathogens onto the keys, and achieve good recovery of bacteria from the keys for enumeration. Results observed during this study are summarized above. In conclusion, UV Angel treatment of C. difficile spores on the keys of both external keyboards and notebooks were effectively killed over time, with the kill increasing with duration of exposure. Similar spore kill was achieved for 15 continuous minutes of exposure (98.57%) and 15 minutes of interrupted exposure (97.29%).

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