

This study was sponsored by CleanLight Technologies, LLC



Effectiveness Testing of the HealthGuard UVC® Bioreduction Device against *Clostridium difficile*, *Staphylococcus aureus*, and *Acinetobacter baumannii*

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Microbac Laboratories, Inc. (Wilson Division) is an FDA registered establishment and is cGMP compliant. Microbac is an ISO 17025:2005 accredited (A2LA) facility.

Throughout, methods marked with * are listed on the accredited scope of work (ISO 17025:2005). All records will be retained in accordance with Microbac SOP ZQAP-097.

INTRODUCTION

The objective of this protocol is to determine the ability of the *HealthGuard UVC® Bio-reduction System* to kill/decrease the concentration of *Clostridium difficile*, *Staphylococcus aureus*, and *Acinetobacter baumannii* with a 60-second and a 120-second exposure time.

TEST ORGANISMS

- C *S. aureus* ATCC 6538
- C *A. baumannii* NCIMB 12457
- C *C. difficile* ATCC 70057

Acinetobacter baumannii

This gram-negative bacteria from a group of microbes found in soil and water has become a formidable nosocomial pathogen associated with significant morbidity and mortality. Able to survive for up to five months on inanimate surfaces, *A. baumannii* can cause necrotizing fasciitis, and is often resistant to many commonly prescribed antibiotics. According to a study published in the February, 2012 issue of the *American Journal of Infection Control*, in survey responses from 822 hospital-based members of the Association for Professionals in Infection Control and Epidemiology, *A. baumannii* was found to be responsible for 13.7 percent of outbreaks.

Staphylococcus aureus

Staph A. outbreaks in healthcare facilities can result in bacteremia or sepsis, pneumonia, endocarditis, and osteomyelitis. While approximately 30 percent of people carry the microorganism in their noses, staph outbreaks can be serious and even fatal in patients with weakened immune systems, those who have undergone surgery and those with intravenous catheters. Staph bacteria can develop resistance to certain antibiotics, which include methicillin-resistant staphylococcus aureus (MRSA), Vancomycin-intermediate staphylococcus aureus (VISA), and Vancomycin-resistant staphylococcus aureus (VRSA).

Clostridium difficile

This pervasive bacterial endospore can be transferred to patients mainly via the hands of healthcare personnel who have touched a contaminated surface or item. *C. diff.* infections are often related to the use of antibiotics, and can cause diarrhea and more serious intestinal conditions such as pseudomembranous colitis. EPA-registered disinfectants with a sporicidal claim have been used for environmental surface disinfection in those patient-care areas where surveillance and epidemiology indicate ongoing transmission of *C. difficile*. In recent years, states have reported increased rates of *C. diff.* outbreaks with an increased rate of mortality.

PROCEDURE

Spike Organism Preparation

Inoculate TSA plates with *S. aureus* and incubate for 22-26 hours at 30-35° C.

Inoculate TSA plates with *A. baumannii* and incubate for 22-26 hours at 30-35° C.

Inoculate RCM+Agar plates with *C. difficile* and incubate for 46-52 hours at 30-35° C in an anaerobic chamber containing an AnaeroGen pak.

Spike organism dilution, plating, incubation, and calculation

Prepare serial dilutions of each culture in 7.2 buffer. Plate 0.1 mL of 10³ CFU/ml concentrations of each organism in duplicate. Plate and incubate as described above. Calculate the concentration of spike organism by multiplying the count acquired by 10 due to the 10⁴ CFU/mL dilutions being used for dilution.

Spiking of pre-poured plates

Inoculate 18 TSA plates with 0.1mL of 10^4 CFU/mL of *S. aureus* and spread with sterile hockey stick

Inoculate 18 TSA plates with 0.1mL of 10^4 CFU/mL of *A. baumannii* and spread with sterile hockey stick

Inoculate 18 TSA plates with 0.1mL of 10^4 CFU/mL of *C. difficile* and spread with sterile hockey stick

UV treatment of spiked plates 60-Second Test

Placed organism-spiked plates inside the HealthGuard UVC® chamber as follows:

Top shelf – right front, back middle and left back

Middle shelf – right back, middle and left middle

Bottom shelf – right middle, middle and left front

Remove lids from plates and set the plates agar side up. Turn unit on and let run for 60 seconds. After treatment period, replace lids on plates. Repeat the above-listed steps for each organism.

120-Second Test

Placed organism-spiked plate inside the HealthGuard UVC chamber as follows:

Top shelf – right front, back middle and left back

Middle shelf – right back, middle and left middle

Bottom shelf – right middle, middle and left front

Remove lids from plates and set the plates agar side up. Turn unit on and let run for 120 seconds. After treatment period, replace lids on plates. Repeat the above-listed steps for each organism.

Incubation of UV-treated plates

Incubate *S. aureus* and *A. baumannii* plates at 30-35°C for 44-52 hours.

Incubate *C. difficile* plates at 30-35°C for 44-52 hours in an anaerobic jar containing an AnaeroGen pak. Note: large jars required three AnaeroGen paks and small jars required one AnaeroGen pak.

ACCEPTANCE CRITERIA The organism spike count from untreated plates served as a positive control to confirm that TSA and RCM+Agar will support correct bacterial growth. An un-spiked TSA plate and an un-spiked RCM+Agar plate will also be incubated to confirm that the plates were not contaminated.

REPORTING

Percent Kill – (a) The percentage-killed of each organism will be determined by dividing the count after the 60-second UV treatment by the original concentration of the organism and then multiplying by 100. (b) For the 120-second test, the percentage-killed of each organism will be determined by dividing the count after the 120-second UV treatment by the original concentration of the organism and then multiplying by 100.

Log₁₀ Reduction – Organism counts will be converted to a log₁₀ number. For example, 213 CFU/mL will equal 2.33 Log₁₀. The Log₁₀ decrease of each organism for the two tests will be determined by calculating the difference between the Log₁₀ count of the original concentration of the organism and the Log₁₀ count after the 60-second and 120-second exposure to UV.

RESULTS

<u>60-Second Treatment</u>	<u>Percent Kill Score</u>
Acinetobacter baumannii	100%
Staphylococcus aureus	100%
Clostridium difficile	100%
	<u>Log₁₀ Reduction</u>
Acinetobacter baumannii	Log _{6.15}
Staphylococcus aureus	Log _{5.80}
Clostridium difficile	Log _{4.0}
<u>120-Second Treatment</u>	<u>Percent Kill Score</u>
Acinetobacter baumannii	100%
Staphylococcus aureus	100%
Clostridium difficile	100%
	<u>Log₁₀ Reduction</u>
Acinetobacter baumannii	Log _{6.15}
Staphylococcus aureus	Log _{5.80}
Clostridium difficile	Log _{4.0}

ABOUT UVC LIGHT

The 1903 Nobel Prize for Medicine was awarded to Niels Finsen for his use of ultraviolet germicidal light (UVC) on tuberculosis. In the 1930s, Westinghouse pioneered the use of UVC lamps in hospital operating rooms. At a certain wavelength, 254 nm, UVC breaks the molecular bonds inside microbial DNA, producing deadly *thymine dimers*, essentially, destroying the germ.

UVC light can eliminate E.coli, MRSA, H1N1, SARS, *pseudomonas aeruginosa*, and many other pathogenic microorganisms. In studies going back more than 70 years, ultraviolet germicidal light (UVC) like that used in HealthGuard UVC® has been shown to kill more than 300 different germs, including bacteria, viruses, mold, fungi, and yeast. There is no microorganism on earth that is resistant to ultraviolet germicidal light.

THE HEALTHGUARD UVC® BIOREDUCTION SYSTEM

Designed for hospital nursing stations, ICUs, clinics, medical offices, etc., *HealthGuard UVC®* is a cart- or countertop-mounted, easy-to-operate, ultraviolet germicidal bio-reduction system that, in 60 seconds, can disinfect patient-contact items like glucometers, stethoscopes, electronic thermometers, blood pressure cuffs, and oximeters, as well as doctor and staff-carried items like cell phones, eMARs scanners, penlights, scissors, PDAs, tablets, laptops, and other easily contaminated, and difficult-to-disinfect items.

HealthGuard UVC® is a new application for proven technology. For the first time, healthcare workers outside of the operating room have access to UVC light's "gold standard" of infection control. *HealthGuard UVC* features a mirror-polished aluminum chamber with 97 percent reflectivity. A cambered interior design helps to 'bend' reflected UVC light waves, reducing typical UVC exposure time while significantly increasing germicidal effectiveness.

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