Effectiveness Testing of the Health Guard UVC Device Against Clostridium difficile, Staphylococcus aureus, and Acinobacter baumannii

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1. Introduction

The objective of this protocol is to determine the ability of the Health Guard UVC to kill/decease the concentration of Clostridium difficile, Staphylococcus aureus, and Acinetobacter baumannii with a 60 second and a 120 second exposure time.

2. Methodology
NA

3. Equipment (as stated below or equivalent)

Incubator at 30-35°C.
Anaerobic jar
Pipettor
Pipet tips
Sterile petri plates
Timer
Sterile cell spreader
Sterile Test tubes

4. Materials (as stated below or equivalent)

AnearoGen Pak ™
Tryptic Soy Agar (TSA)
Reinforced Clostridial Medium + Agar (RCM+Agar)
7.2 pH buffer

5. Test organisms

S. aureus ATCC 6538 from Microbiologics
A. baumannii NCIMB 12457 from Microbiologics
C. difficile ATCC 70057 from Microbiologics

6. Procedure

6.1 Spike organism preparation
- 6.1.1 Inoculate TSA plates with S. aureus and incubate for 22-26 hours at 30-35°C.
- 6.1.2 Inoculate TSA plates with A. baumannii and incubate for 22-26 hours at 30-35°C.
- 6.1.3 Inoculate RCM+Agar plates with C. difficile and incubate 46-52 hours at 30-35°C in an anaerobic chamber containing an AnaeroGen pak.

6.2 Spike organism dilution, plating, incubation, and calculation

Prepare serial dilutions of each culture in 7.2 buffer. Plate 0.1 mL of 10^3 CFU/mL concentration of each organism in duplicate. Plate and incubate as described in section 6.1. Calculate the concentration of the spike organism by multiplying the count acquired by 10 due to the 10^4 CFU/mL dilution being used for spiking.

6.3 Spiking of pre-poured plates

6.3.1 Inoculate 18 TSA plates with 0.1 mL of 10^4 CFU/mL of S. aureus and spread with sterile hockey stick.
6.3.2 Inoculate 18 TSA plates with 0.1 mL of 10^4 CFU/mL of A. baumannii and spread with a sterile hockey stick.
6.3.3 Inoculate 18 RCM+Agar plates with 0.1 mL of 10^4 CFU/mL of C. difficile and spread with a sterile hockey stick.

6.4 UV treatment of spiked plates

6.4.1 Place organism spiked plate in Health Guard UVC as follows:
Top shelf- right front, back middle and left back
Middle shelf-right back, middle and left middle
Bottom shelf-Right middle, middle, left front
6.4.2 Remove lids from plates and set the plates agar side up.
6.4.3 Turn on the unit and let run for 60 seconds
6.4.4 Turn off the unit and replace lids on plates
6.4.5 Repeat steps 6.4.1 through 6.4.4 for each organism
6.4.6 Place organism spiked plate in Health Guard UVC as follows:
Top Shelf-Middle front, left back, Right back
Middle Shelf-Middle back, middle, left front
Bottom Shelf-Right front, middle front, left back
6.4.7 Remove lids from plates and set the plates agar side up
6.4.8 Turn on the unit and let run for 120 seconds.
6.4.9 Turn off the unit and replace lids on plates.
6.4.10 Repeat steps 6.4.6 through 6.4.9 for each organism.

6.5 Incubation of UV treated plates.

6.5.1 Incubate S. aureus and A. baumannii plates at 30-25°C for 44-52 hours.
6.5.2 Incubate C. difficile plates at 30-35°C for 44-52 hours in an Anaerobic jar containing an AnaeroGen pak. Note: large jars require three AnaeroGen paks and small jars require one AnaeroGen pak.

7. Acceptance criteria

The organism spike count from section 6.2 above will serve as a positive control to confirm that TSA and RCM+Agar media will support the correct bacterial growth. A un-spiked TSA

plate and an un-spiked RCM+Agar plate will also be incubated with the plates from section 6.2 to confirm that the plates are not contaminated.

8. Reporting of percent kill of each organism for each exposure time

   8.1 Calculate the percent kill of each organism by dividing the count after the 60 min. exposure to UV by the original concentration of the organism (section 6.2) and then multiplying by 100.

   8.2 Calculate the percent kill of each organism by dividing the count after the 120 min. exposure to UV by the original concentration of the organism (section 6.2) and then multiplying by 100.

9. Reporting of Log_{10} reduction of each organism for each exposure time

   9.1 Convert each organism count to a log_{10} number. For example 213 CFU/mL will equal 2.33 Log_{10}.

   9.2 Calculate the Log_{10} decrease of each organism for each exposure time by calculating the difference between the Log_{10} of the original concentration of the organism (section 6.2) and the Log_{10} after the 60 and 120 min. exposure to UV.