**A do-it-yourself test protocol using commercial *Bacillus atrophaeus* spores to evaluate the effectiveness of ultraviolet-C light room decontamination devices**

**Scope**

This protocol provides test conditions to evaluate the effectiveness of ultraviolet-C (UV-C) light room decontamination devices. The *Bacillus atrophaeus* spore preparations used for testing are purchased from commercial laboratories with expertise in production of biological indicators. The test protocol is intended to provide a standard method that infection prevention or environmental services personnel can use to evaluate the effectiveness of in-use UV-C devices or compare effectiveness of UV-C devices being considered for purchase. The protocol can be completed with no requirement for on-site microbiological expertise if pre-prepared disks are purchased and sent back to the commercial laboratory for testing. Alternatively, disks can be processed on-site using methods that are commonly available in clinical or research microbiology laboratories. The standard protocol specifies that carriers are placed .914 meters (3 feet) from the device and exposed to UV-C for 10 minutes. However, the same carriers could be used to evaluate longer cycle times, different distances from the device, and sites in patient rooms that are not directly exposed to UV-C.

**Reagents and Materials**

*Test organism*: The recommended indicator is *Bacillus atrophaeus* (ATCC 9372) spores purchased from Mesa Laboratories (Lakewood, CO) as spore suspensions (CAT# SSGE/7) or on pre-prepared 8x12 mm steel disks inoculated with 103 or 106 colony-forming units (CFU) of spores (CAT#’s GRS-090E3 & GRS-090) (**Picture 1**)

*Petri Dishes*: 100x15 mm, sterile, plastic

*2-sided tape*: to adhere disks to petri dishes

*Sterile 10-15-mL tubes*: to place disks into after UV-C exposure

*Forceps*: to transfer steel disks (disposable or sterilized between disks)

*Flat, vertical surface to adhere petri dishes*

*Tape to adhere petri dish to surface*

*UV-C room decontamination device*

*Tape measure or ruler*

*Ice packs*

*Sterile water*

***Optional items needed only if spore suspensions are used instead of pre-pared disks***

*Pipettor:* capable of a volume up to 1 mL and a precision of 0.001 mL

*Sterile disposable 10 µL inoculating loops*: alternative to pipettor for inoculation of steel disks

*Steel disks*: 10 mm in diameter

***Optional items needed only if cultures will be processed on site***

*Growth media*: trypticase soy broth and trypticase soy agar plates

*Incubator*: set at 30°C

*Sterile 15 mL tubes*: to hold disks

*Sterile deionized water*

*6 mm glass beads*: 4

*Vortex mixer*

**Test procedures**

***Protocol 1***. Reduction in *B. atrophaeus* using 106 CFU steel disks

1). A minimum of six carriers are needed including

3 to be exposed to UV-C treatment

3 to be untreated controls.

2). Apply 2-sided tape to the surface of the Petri dishes.

3). Using sterile forceps for each disk, adhere the disks to the tape. The concave side with the *B. atrophaeus* spore inoculum should face outward toward the UV-C device (**Picture 2**).

4). Use tape to adhere the petri dishes to the vertical surface at 0.91 meters from the bulb at a height that is at the mid-point of the bulb height (**Picture 3**). Take care not to obstruct the device UV light with tape.

5). Operate the UV-C device to provide a 10-minute UV-C exposure.

6). For each carrier, use sterile forceps to transfer treated carriers to a separate tube and to transfer untreated carrier to a separate tube.

7). Process cultures on-site as described under Microbiology or package the tubes on ice packs with packing material and mail to the commercial test lab for quantitative cultures.

8). Record the type of device being tested as well as the model number and bulb life.

9). Record the temperature and relative humidity measurements at the beginning and end of the exposure period.

***Protocol 2***. Reduction in *B. atrophaeus* on 103 CFU steel disks to undetectable levels

1). A minimum of thirteen carriers is recommended including:

10 exposed to UV-C treatment

3 untreated controls.

2). Apply 2-sided tape to the surface of the Petri dishes.

3). Using sterile forceps for each disk, adhere the disks to the tape. The concave side with the *B. atrophaeus* spore inoculum should face outward toward the UV-C device (**Picture** 1 **and 2**).

4). Use tape to adhere the petri dishes to the vertical surface at 0.914 meters from the bulb at a height that is at the mid-point of the bulb height. Take care not to obstruct the light from the device with tape (**Figure 1)**

5). Operate the UV-C device to provide a 10-minute UV-C exposure.

6). If the disks are from a commercial lab, use sterile forceps to flip each disk over and re-adhere to a new piece of tape (**Picture 4**) followed by administering a second 10-minute cycle of UV-C. The purpose of the second UV-C exposure is to kill any spores contaminating the non-inoculated side of the disk; in preliminary experiments, we have demonstrated that low levels of spore contamination may be present on the non-inoculated side of the disk resulting in false-positive broth-enrichment cultures (**Picture 5**).

7). For each carrier, use sterile forceps to transfer treated carriers to a separate tube and to transfer untreated carrier to a separate tube

7). Package the tubes on ice packs with packing material and mail to the commercial test lab for qualitative cultures or process cultures on-site as described under Microbiology. No special shipping is required as no hazardous materials are included in the package.

***Protocol 3***. Preparation of 106 or 103 CFU *B. atrophaeus* disks if pre-prepared disks are not used

1). For preparation of 106 CFU disks, use a pipettor to pipette 10 *µL* of *B. atrophaeus* spore suspension (107 CFU per 0.1 mL) onto the center of a 10mm steel disk. If a pipettor is not available a 10µl inoculating loop can be used.

2) For preparation of 103 CFU disks, use a pipettor to dilute spore suspension (107 log per 0.1 mL) 1:1000 by adding 1µl of the spore suspension to 999µl of sterile water. Use a pipettor to pipette 10 µL of diluted *B. atrophaeus* spore suspension onto the center of a 10mm steel disk. If a pipettor is not available a 10µl inoculating loop can be used.

3). Allow the droplet to dry at room temperature until the droplet is no longer visible (~15 to 30 minutes).

4). The process is repeated for each carrier.

5). Between uses, carriers should be cleaned and sterilized in a manner compatible to steel disks.

***Protocol 4***. Microbiology

A). Reduction in *B. atrophaeus* on 106 CFU steel disks

1). Use sterile forceps to transfer each disk to tubes containing four 6mm glass beads and vortex for no less than 4 minutes in 1 mL of sterile deionized water.

2). Serially dilute the treated and untreated samples in sterile deionized water.

3). Use standard enumerative techniques to quantify the viable population of *B. atrophaeus* recovered from each disk by plating aliquots of the diluted samples onto trypticase soy agar plates.

4). Incubate the plates at 30°C for up to 48 hours. Confirm the identity of *B. atrophaeus* by colony morphology and Gram staining at a minimum. *B. atrophaeus* will appear mauve to pink on trypicase soy agar plates and in trypticase soy broth at 48 hours.

5). Calculate the log10 reduction in spores by subtracting viable organisms recovered from UV-C exposed versus unexposed control carriers. The percent reduction can also be calculated as described in E3135-18.

6). The population assay recovery method recommended by Mesa Labs can be used as an alternative; in preliminary experiments the method described yielded nearly identical results to the population assay recovery method recommended by Mesa Labs (**Figure 2**).

7). The sterility of the carriers and extraction solution should be confirmed.

B).Reduction in *B. atrophaeus* on 103 CFU steel disks to undetectable levels

1). Use sterile forceps to transfer each disk to tubes containing 2 mL of trypticase soy broth.

2). Incubate the tubes at 30°C for up to 48 hours.

3). For tubes demonstrating turbidity (cloudy media) (**Picture 6**), plate 10 *µL* on trypticase soy agar and incubate at 30°C for up to 3 days to assess for growth. For tubes that do not demonstrate turbidity and for tubes with turbidity that do not initially culture positive for *B. atrophaeus*, plate 10 *µL* on trypticase soy agar after 7 days of incubation. On trypticase soy agar, confirm growth of *B. atrophaeus* based on typical colony appearance. *B. atrophaeus* will appear mauve to pink on TSA plates and in TSA broth at 48 hours.

4). Calculate the percentage of tubes with growth of *B. atrophaeus*.

**Figures**

**Figure 1**. Illustration of the setup for testing. Disks were adhered to petri dishes in parallel to the lamps at the mid-point of the bulb height.



**Figure 2**. Comparison of recovery of *Bacillus atrophaeus* from UV-C exposed versus unexposed steel disks using the recommended test protocol versus the population assay recovery method used by Mesa Laboratories



**Pictures**

**Picture 1.** *Bacillus atrophaeus* spores obtained from Mesa Laboratories







**Picture 2**. Steel disks adhered to a petri dish with tape. The disks are attached to the tape using forceps with the inoculated side of the disks faces toward the UV-C device. The disks are offset on the tape for easy removal after UV-C exposure.



**Picture 3**. Steel disks oriented vertically in parallel to the UV-C device bulbs



**Picture 4**. Use of forceps to flip the disks over and re-adhere to the tape prior to a second 10-minute UV-C cycle. This procedure is indicated for the evaluation of reduction in *B. atrophaeus* on commercial 103 CFU steel disks to undetectable levels. The rationale for providing a second UV-C cycle is to eliminate small numbers of spores that may inadvertently contaminate the non-inoculated side of the disk.



**Picture 5**. Trypticase soy agar plate showing *Bacillus atrophaeus* spore contamination on the non-inoculated side of a 103 CFU *B. atrophaeus* commercial disk obtained from Mesa laboratories. The disk was aseptically removed from the packaging, imprinted onto the surface of the culture plate, and incubated at 30°C for 48 hours.



**Picture 6**. Broth enrichment cultures of UV-C exposed or unexposed disks with 103 CFU of *Bacillus atrophaeus*. All tubes were processed to assess for growth of *B. atrophaeus* based on typical colony appearance and Gram stain.



Positive (unexposed control)

Negative (after UV-C exposure)

Positive (unexposed control) vortexed