



# WHITE PAPER

## 3B Lumin and Disinfection of CPAP Accessories

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 **3B<sup>TM</sup> Medical, Inc.**

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## Introduction – Applied Optics of UVC for Disinfection

The human eye can detect electromagnetic radiation in the visible spectrum, which ranges from wavelengths of 400 nm (violet) to 700 nm (red). Since the early 20th century, we have known that light comes in packets called photons, and each photon has an energy  $E = \frac{hc}{\lambda}$ , where  $c = 3.0 \times 10^8$  m/s is the speed of light,  $h$  is a constant known as Planck's constant, and  $\lambda$  is the wavelength of the light. This equation means that the wavelength and energy of electromagnetic radiation are

inversely proportional: a shorter wavelength means a higher energy, which generally corresponds to a greater possibility of damage to living organisms.

Ultraviolet radiation is defined as radiation with a wavelength of less than 400 nm and greater than 100 nm, so it is below the visible spectrum and thus not detectable by the human eye. Ultraviolet radiation is split into 3 subdivisions, as shown in the following table.

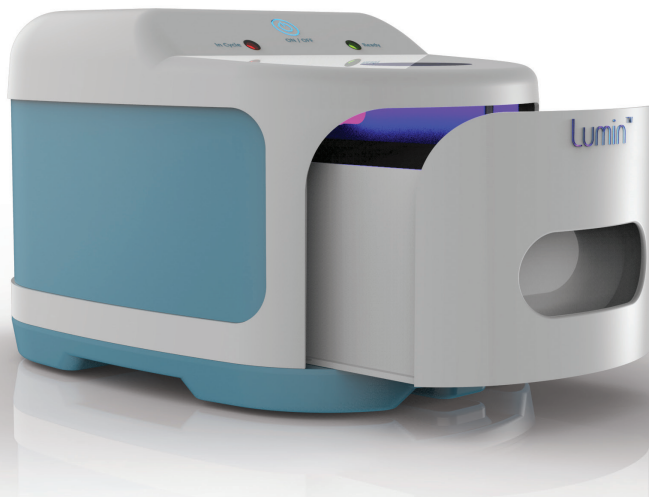
Classification	Wavelengths
Ultraviolet A (UVA)	400nm – 315nm
Ultraviolet B (UVB)	315nm – 280nm
Ultraviolet C (UVC)	280nm – 100nm

UVA is used in tanning beds and black lights, and 97% of ultraviolet radiation that reaches the Earth's surface is in this category. UVB causes sunburns, and accounts for 3% of the ultraviolet radiation at the Earth's surface<sup>1</sup> UVC radiation from the Sun is absorbed by the atmosphere and does not reach the Earth's surface. UVC radiation is also absorbed by ordinary glass, and reflected by metallic conductors such as aluminum.

Ultraviolet radiation is very harmful to biological microorganisms, such as viruses and bacteria. It interacts with these organisms by altering base pairing in their DNA and RNA and thus preventing them from reproducing. Ultraviolet radiation has been used in medicine since the work of Niels Finsen in the late 1800s. The Nobel Prize in Medicine and

Physiology was awarded to Finsen in 1903. It has been known since 1937 that for germicidal applications, the wavelength of maximum effectiveness is near 260 nm<sup>2</sup>.

Conveniently, low-pressure mercury vapor lamps have an emission spectrum peaked at 254 nm, so these lamps have been used in germicidal applications for quite some time. The mechanism behind this type of lamp is ionized mercury vapor allowing an electrical arc to pass between two electrodes. The lamp used in the Lumin is a low-pressure mercury vapor lamp with a power of 2.3 W in the UVC range, concentrated at a wavelength of 254 nm. The lamp is made using quartz glass for the bulb, which allows UVC radiation to pass through.



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<sup>1</sup> Brian L. Diffey. What is light? *Photodermatology, Photoimmunology, and Photomedicine*, 18(2):68-74, 2002.

<sup>2</sup> D. Gordon Sharp. A quantitative method of determining the lethal effect of ultraviolet light on bacteria suspended in air. *Journal of Bacteriology*, 35(6):589-599, 1938.

## Calculation of Lumin's Disinfection Power

It is instructive to perform an order-of-magnitude calculation of the irradiance provided by the Lumin, in order to help determine the distance and dosage needed for various levels of disinfection.

As a zeroth-order approximation, a radiation source such as a lamp or a lightbulb can be treated as a point source. The electromagnetic radiation from the source then falls off as  $1/r^2$ , where  $r$  is the distance to the source (the inverse-square law). However, this approximation tends to break down when the distance between the source and the detector is less than five times the greatest dimension of the source<sup>3</sup>. In the case of the Lumin, the greatest dimension is the length of the lamp,

which is 127.2 mm (neglecting the base). So a detector would need to be located at distances greater than about 60 cm in order for the point source approximation to hold. The disinfection chamber is only 20 cm deep, so a more detailed calculation is necessary.

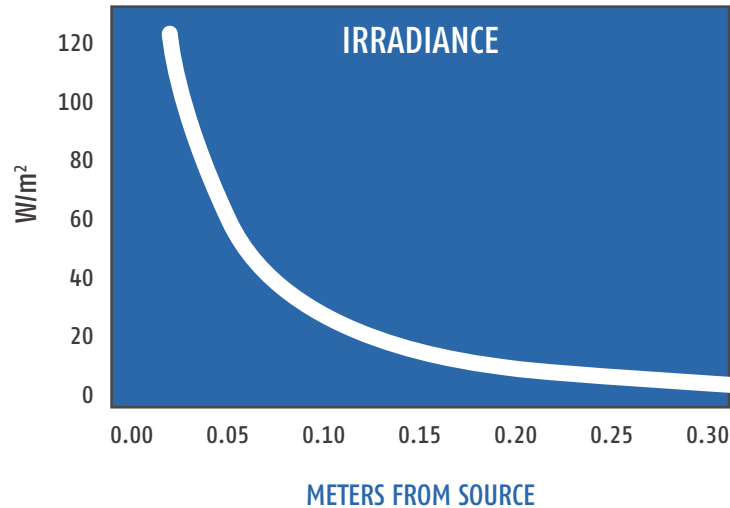
Since the UVC lamp used in the Lumin is about 4.5 times longer than it is wide, the next-best approximation to use is the linear source approximation. This treats the lamp as a one-dimensional source of length  $L$ , made up of an array of point sources. The irradiance at a distance  $d$  from the linear source for a detector located a distance  $h$  from one end of the source is<sup>4</sup>

$$E(h, d) = S_R \left( \frac{L-h}{d\sqrt{d^2 + (L-h)^2}} + \frac{h}{d\sqrt{d^2 + h^2}} \right)$$

Here  $S_R = \frac{\phi}{4\pi r^2 L}$ , where  $\phi = 2.3 \text{ W}$  is the intensity at the desired UVC wavelength (254 nm). For a point located at the midpoint of the lamp ( $h = L/2$ ) at a distance  $d = 4 \text{ cm}$  from the lamp, the above equation gives an irradiance of  $7.75 \text{ mW/cm}^2$ . For comparison, a brochure produced by the manufacturing company (Philips) shows an irradiance of approximately  $9.1 \text{ mW/cm}^2$  at a distance of  $4 \text{ cm}$  from the bulb<sup>5</sup>. So using a linear source

approximation in the form of the above equation gives a conservative estimate of the irradiance. The discrepancy is most likely due to the breakdown of the linear source approximation at small distances from the lamp. Figure 1 shows a plot of the irradiance given by the above equation in units of Watts per square meter as a function of distance from the lamp.

Irradiance is a useful quantity, but for disinfection purposes it is more useful to talk about the dosage, obtained by multiplying irradiance by exposure time.



These curves represent a conservative estimate of the dosage at these relatively low distances from the UVC lamp.

Figure 1. UVC irradiance as a function of distance from the lamp.

Irradiance is a useful quantity, but for disinfection purposes it is more useful to talk about the dosage, obtained by multiplying irradiance by exposure time. The results of calculating dosage for 5 and 10-minute exposure times are shown in Figures 2 and 3. The horizontal red dashed line shows the dosage necessary for a log 2 reduction in *Aspergillus niger*, which is  $330.0 \text{ mJ/cm}^2$  (*Aspergillus niger* is one of the hardest molds). This dosage was the highest listed in a comprehensive dosage table for various spores, bacteria, yeasts, molds, protozoa, and viruses<sup>6</sup>.

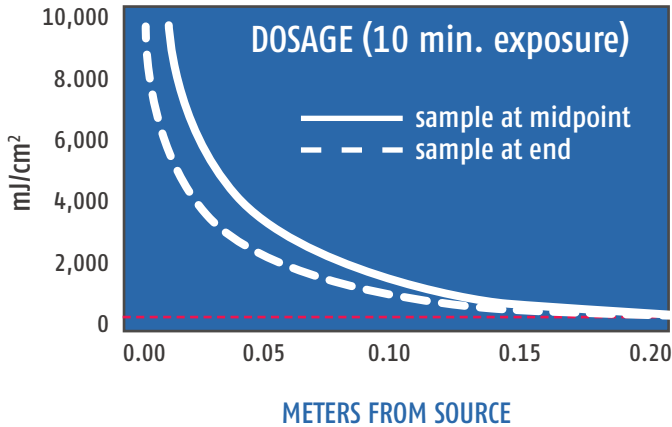
A log 2 reduction means that there is a 99% reduction of organisms at this dosage. Two dosage curves are shown, one (the solid curve) for a sample directly across from the midpoint of the lamp, and the other (the dashed curve) for a sample directly across from one end of the lamp. This illustrates the small variation in irradiance for samples not placed directly under the center of the lamp. Again, recall that these curves represent a conservative estimate of the dosage at these relatively low distances from the UVC lamp.

<sup>3</sup> J. Murdoch. Illumination Engineering – From Edison's Lamp to the Laser. Collier Macmillan, New York, 1985.

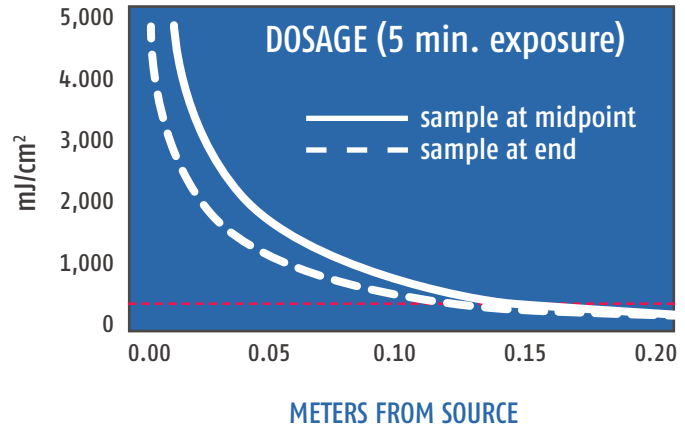
<sup>4</sup> David Robert Grimes, Chris Robbins, and Neil John O'Hare. Dose modeling in ultraviolet phototherapy. *Medical Physics*, 37(10):5251-5257, 2010.

<sup>5</sup> Koninklijke Philips Electronics N.V. Ultraviolet purification application information. 2006.

<sup>6</sup> ClorDiSys. Ultraviolet light disinfection data sheet. 2013.



**Figure 2.** Dosage curves for the Lumin for a 10-minute exposure time. The horizontal red dashed line represents a dosage of 330.0 mJ/cm<sup>2</sup>, necessary for a log 2 reduction in *Aspergillus niger*.



**Figure 3.** Dosage curves for the Lumin for a 5-minute exposure time. The horizontal red dashed line represents a dosage of 330.0 mJ/cm<sup>2</sup>, necessary for a log 2 reduction in *Aspergillus niger*. Note the different scale on the y-axis as compared to Figure 2.

Now, the lamp is actually composed of two cylinders, so it would be possible to more precisely model the irradiance using a significantly more complicated model, along the lines of those outlined in Section 1.5.2 of<sup>7</sup>. However, since the model provided by the above equation matches up closely with the irradiance values provided in [5], and since we are just looking for an approximate calculation, using a more complicated model reaches the point of diminishing returns.

However, there is one important aspect of the Lumin that has not yet been taken account of in this calculation. That is the fact that the box is made out of polished aluminum. Polished aluminum is highly reflective for UV wavelengths of radiation. Specifically, the aluminum used for the Lumin has a #8 mirror finish with a UVC reflectivity of 80%.

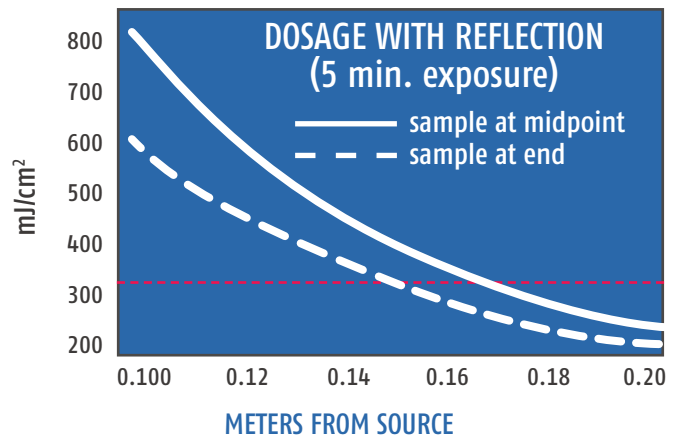
### The Lumin's box is polished aluminum, which is highly reflective for UV wavelengths of radiation.

(The aluminum used for the Lumin has a #8 mirror finish with a UVC reflectivity of 80%.)

### This reflectivity will significantly increase the UVC radiation incident upon a sample placed under the lamp.

As a conservative estimate, we can take a 40% increase in irradiance at points under the lamp (central points) due to the reflection provided by the sides of the Lumin. For a 10-minute exposure time, this results in a dosage greater than 330.0 mJ/cm<sup>2</sup> for all points under the lamp and within the confines of the Lumin. For a 5-minute exposure time, all points under the lamp and within 15 cm of the lamp receive a dosage greater than 330.0 mJ/cm<sup>2</sup>. Figure 4 shows the result including the estimate for the reflectance contribution for a 5-minute exposure time and distances ranging from 10 to 20 cm from the lamp.

This reflectivity will significantly increase the UVC radiation incident upon a sample placed under the lamp. Obtaining a precise number for the increase in irradiance would require a detailed ray-tracing study using commercially available software, but similar research has been done on modeling the increase in irradiance due to reflectivity for ultraviolet phototherapy<sup>8</sup>. In this study, the reflectivity was 80% and resulted in an average increase in peak irradiance of 130% (the irradiance was more than doubled for central points)! Now, the geometry in [8] is slightly different, since the side mirrors in that case are at an angle. But further research found that smaller angles for the side mirrors decreased the overall reflectance contribution but actually increased the peak irradiance slightly<sup>9</sup>.



**Figure 4.** Dosage curves for points under the lamp for a 5 minute exposure time including the estimated increase in irradiance due to reflection. The plot is for points ranging from 10–20 cm from the lamp. The horizontal red dashed line is again at a dosage of 330.0 mJ/cm<sup>2</sup>.

<sup>7</sup> M.W. Burke. *Image Acquisition: Handbook of machine vision engineering*. V. 1. Springer Netherlands, 2012.

<sup>8</sup> David Robert Grimes, Chris Robbins, Colin J. Martin, Graeme Phanco, and Neil John O'Hare. Reflection modeling in ultraviolet phototherapy. *Medical Physics*, 38(7):4312–4320, 2011.

<sup>9</sup> David Robert Grimes, Colin J Martin, and Graeme Phanco. Investigations of cabin design in uv phototherapy. *Medical Physics*, 39(6Part1):3019–3025, 2012.

The conclusion that can be drawn from the preceding calculation and estimate is that the Lumin is a highly effective device for delivering large dosages of germicidal UV radiation in relatively short exposure times.

## Part II: Microbiology and Lumin's Disinfection Ability

### Introduction

Microbes are ubiquitously distributed; in other words, microbes such as bacteria, viruses, yeasts and molds inhabit all kinds of environments. To obtain and maintain inhabitation in these various environmental conditions, cells have to evolve. For example, thermophiles thrive at extreme temperatures, such as those found at deep ocean thermal vents. One trait that has evolved in thermophiles is the guanine and cytosine (GC) content of the organism. This trait keeps the DNA "intact" at high temperatures because there are three hydrogen bonds between G and C and only two between adenine (A) and thymine (T), therefore requiring more energy to break the hydrogen bonds that link the two strands of DNA.

Another trait that has evolved in some species is the formation of a spore. Spores are an effective way to "hibernate", under less than ideal environmental conditions. Some bacterial species have a cell signaling pathway that effectively decrease cellular metabolism and triggers the formation of a spore. The resulting spores are resistant to nearly all environmental fluctuations, as well as some sterilizing methods. It is important to know, while longevity of the spore is variable, spores

do not represent dead cells because once environmental cues indicate optimal growth conditions the spore germinates and is fully functional. *Geobacillus stearothermophilus* fits into both of these categories. These characteristics make it a good candidate to be used as a bioindicator (an organism that is used as a standard for various tests, in this case to determine parameters of UVC induced killing) because this species is relatively robust. For example, it is resistant to toxic oxidizing agent potassium tellurite<sup>10</sup> and it is sensitive to ozone<sup>11</sup> just to name a few. In other words, *G. stearothermophilus* cannot be killed using some sterilization techniques due to spore formation and its thermophilic growth temperature. So, if *G. stearothermophilus* is killed by 3B Lumin it stands to reason that most other organisms present would also be killed as they are more sensitive to the 3B Lumin due to physical characteristics just described.

Due to the characteristics of spores (as mentioned above) care, diligence and close monitoring need to be considered during sterilization techniques.

### Briefly, a few common methods include<sup>12</sup>:

<p>1. Autoclave which is the primary method of sterilization, it entails the use of a pressurized chamber, high temperature (generally 121°C) and steam. Duration is dependent on the primary target, but 20 minutes is standard to kill spores. Although this technique is known to kill spores, the parameters need to be adjusted accordingly. Due to the mechanics of autoclaving it is not very practical to have an in-home system.</p>	<p>2. Dry heat is an alternative to autoclaving that lacks steam. Due to the lack of steam duration and/or heat need to be adjusted. This method is also effective in killing spores, however the temperature should be increased to 180°C and the time should be increased to 60 minutes. Like autoclaving, dry heat is not practical as a home use technique.</p>	<p>3. Filtration is a viable alternative, however, it is only effective for liquids. This rapid system has two limitations, first it only works on liquids, second the standard filter size (pore size) is not effective at removal of viruses and phages due to their small size.</p>	<p>4. The most common liquid methods utilize ethanol or Isopropanol diluted to 60-90%. Potassium tellurite [1] is another chemical used for sterilization. These chemicals are effective especially to clean work areas. This is a "quick and dirty" sterilization technique, however it is not effective for spores.</p>	<p>5. Radiation, specifically UV treatment is an example of radiation treatment that is used for sterilization. UV can penetrate the cell resulting in DNA damage that is too extensive to be repaired via cellular DNA damage repair pathways thus resulting in cellular death. - Due to the relative rate and efficacy of UV exposure, specifically UVC, it is the preferred sterilization method for some situations including in home sterilization protocols.</p>
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<sup>10</sup> Tantalean J.C., et al. The *Geobacillus stearothermophilus* V iscS gene, encoding cysteine desulfurase confers resistance to potassium tellurite in *Escherichia coli* K-12. *Journal of Bacteriology*, 185(19):5831-7.

<sup>11</sup> Redigueri C.F. et al. Ozone Gas as a benign sterilization treatment for PLGA. nanobifer scaffolds. *Tissue Engineering. Part C Methods*. 22(4):338-347.

<sup>12</sup> Oswald N. 5 Laboratory sterilization methods. Accessed 12/05/2017 <https://bitesizebio.com/853/5-laboratory-sterilisation-methods/subterranean-gen-nov-sp-nov-and-geobacillus-uzenensis-sp-nov-from-petroleum-reservoirs-and-transfer-of-bacillus-stearothermophilus-bacillus-thermocatenulatus-bacillus-thermoleovorans-bacillus>

To test the efficacy of any of these methods a bioindicator (a microbe that is used as a representative organism to test the effectiveness of a particular method) is exposed to the sterilization method being tested. In this case UVC exposure was tested using *G. stearothermophilus* as the bioindicator. As indicated in the previous section, *G. stearothermophilus* is both a thermophile<sup>13</sup> and a spore former<sup>14</sup>. These properties alone physically make it more resistant to UVC irradiation. *G. stearothermophilus*

spores were shown to be sensitive to autoclaving<sup>15</sup> indicating that these spores are sensitive to a commonly used sterilization process, it is important to have this positive control to ensure the sample being tested is indeed able to be killed. Also, *G. stearothermophilus* has been reported to be the type species<sup>16</sup>. Taken together these characteristics make *G. stearothermophilus* a suitable biomarker for evaluating the efficacy of UVC sterilization.

### Methods Used in Testing Lumin

*G. stearothermophilus* was used as the bioindicator to determine kill time. Stainless steel carriers from the Apex BI line were used with having been inoculated with a 10<sup>5</sup> CFU *Geobacillus stearothermophilus*. The 3B Lumin device generating UVC at a dose greater than 330.0 mJ/cm<sup>2</sup> was used as the disinfecting agent. Efficacy was tested using two independent products, CPAP water humidifier and a CPAP mask. Briefly, one sample for each product and a negative control were exposed to UVC for 3, 5, or 7 minutes using the 3B Lumin; note the negative control was

not subjected to UVC. Plate counts were used to determine the microbial load of *G. stearothermophilus* from each sample. The protocol used has a lower detection limit of 5 CFU/sample. Stainless steel carriers were inoculated with ~10<sup>5</sup> colony forming units (CFU) then diluted (10<sup>-1</sup>, 10<sup>-2</sup> and 10<sup>-3</sup>) prior to incubation on Tryptic Soy Agar. Samples were incubated at 55–60°C for seven days. This relatively high incubation temperature is in-line with the thermophilic properties of *G. stearothermophilus*.

### Results and Discussion

Data indicate that a dose of 330.0 mJ/cm<sup>2</sup> is effective at killing *G. stearothermophilus* so effective that there was no detectable growth following 3, 5 or 7 minute exposure (Table 1).

Sample ID	Bacteria CFU / Sample
A1 - Lumin - Dose at 3 Min. Exposure	<5.00E + 00
A2 - Lumin - Dose at 3 Min. Exposure	<5.00E + 00
B1 - Lumin - Dose at 3 Min. Exposure	<5.00E + 00
B2 - Lumin - Dose at 3 Min. Exposure	<5.00E + 00
C1 - Lumin - Dose at 3 Min. Exposure	<5.00E + 00
C2 - Lumin - Dose at 3 Min. Exposure	<5.00E + 00
D1 - CONTROL	3.10E + 04
D2 - CONTROL	3.10E + 04

**TABLE 1:**

Data indicating for each sample tested the biomarker was sufficiently killed by UVC at a dose of 330.0 mJ/cm<sup>2</sup>. Testing was conducted by an independent laboratory, Microchem Laboratory.

<sup>13</sup> Aliye H. et al. Phylogenomic re-assessment of the thermophilic genus *Geobacillus*. *Systematic and Applied Microbiology*. 39(8):527–533.

<sup>14</sup> <https://www.ncbi.nlm.nih.gov/mesh/?term=geobacillus> then retrieve the data by selecting species name.

<sup>15</sup> Huesca-Espitia L. C. et al. Effects of steam autoclave treatment on *Geobacillus stearothermophilus* spores. *Journal of Applied Microbiology*. 121(5):1300–1311.

<sup>16</sup> Nazina, T.N., et al. Taxonomic study of aerobic thermophilic bacilli: descriptions of *Geobacillus subterraneus* gen. nov., sp. nov. and *Geobacillus uzenensis* sp. nov. from petroleum reservoirs and transfer of *Bacillus stearothermophilus*, *Bacillus thermocatenulatus*, *Bacillus thermoleovorans*, *Bacillus*

## Results and Discussion (cont'd)

These results indicate that at each time tested there was 100% kill rate. As indicated in the introduction a bioindicator, in this case, is an organism that is relatively resistant to numerous sterilization techniques and thus is used to extrapolate absence of organisms that are more sensitive. For example, we have determined 100% kill rate using the 3B Lumin. This data compared with data presented in Phillips UV technology brochure that identified the dose necessary for 90% kill rate varies tremendously (table 2 is a selective representation of various microbes)<sup>17</sup>. For example all bacterial species tested had a 90% kill rate at doses much lower than the 330.0 mJ/cm<sup>2</sup>, the range was 9 to 197 mJ/cm<sup>2</sup> (of note, this included spore forming strains as well), the same is true for yeast that ranged from 33 to 80 mJ/cm<sup>2</sup>, this trend is extended to viruses and protozoans tested. The fact that the majority of species tested have a much lower dose required for microbial killing indicates we can, with confidence, infer that these organisms have been killed as well, leaving the resulting product sterilized.

Species Name	Description 90%	Kill dose
<i>Bacillus megatherium</i>	Spore former	27.3
<i>Clostridium tetani</i>	Pathogenic agent of tetanus	120
<i>Staphylococcus aureus</i>	Opportunistic pathogen	26
<i>Influenza virus</i>	Causes the flu	36
<i>Saccharomyces cerevisiae</i>	Used in wine making, baking and brewing	60

**TABLE 2:**

Representative microbes and the 90% dose. Meaning at the listed dose 90% of the microbes are killed [8].

A five minute cycle is recommended, to ensure sterilization due to variable factors that can effect the efficacy of 3B Lumin. These parameters include, but are not limited to the size of the disinfection space as well as reflective properties of the metal used for the experiment. Additionally the positioning of the mask and water chamber will effect the results as well. While a 3 minutes exposure resulted in a 100% kill rate, the extended 5 minute exposure allows "wiggle room" during personal use.

## Conclusion

Our tests using 330.0 mJ/cm<sup>2</sup> at three, five and seven minutes all resulted in a 100% kill rate of *G. stearothermophilus*. Compared to a similar test [8] we conclude that most other microbes were also killed. This is based on comparing reported data measuring 90% kill rate at much lower doses (table 2). Taken together we conclude that *G. stearothermophilus* is an effective biomarker for UVC exposure and, more importantly, the 3B Lumin is capable of reaching 100% sterility.



kaustophilus, Bacillus thermodenitrificans to Geobacillus as the new combinations *G. stearothermophilus*, *G. th.. International Journal of Systematic and Evolutionary Microbiology*. 51:433-446.

<sup>17</sup> Koninklijke Philips Electronics N.V. *Ultraviolet purification application information*. 2006.





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