Efficacy of LUMA Bottle in reducing live Escherichia coli populations utilizing time-kill procedure

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Abstract:
The LUMA Bottle claims a >99% reduction of bacterial populations within the contents of the Bottle after 1 minute of continuous ultraviolet light exposure from a low-power UV-C light set into the base of the device. The purpose of this experiment is to measure the efficacy of this claim. Standard antimicrobial time-kill procedure principles were followed, as obtained in *The Standard guide for Assessment of Antimicrobial activity using a Time-Kill Procedure.* The test was performed independently using materials provided by Analytical Resource Labs in Lehi, UT.

Materials and Methods:
One LUMA bottle was provided, from which all tests were conducted. 500 mL of sterile de-ionized water was introduced into the bottle and subsequently plated onto 25 mL Tryptic Soy Agar (TSA) plates in triplicate at the quantity of 1 mL per plate. These were incubated for 48 hours at 35˚C as negative controls. 1 mL from the bottle was also introduced into a sterile 100 mL bag of Tryptic Soy Broth (TSB) and incubated at the same temperature and duration as a further control. We then introduced 500 µL of standard *E. coli* stock titered to 10^4 CFUs, determined using the OD600 reading. After thorough mixing, a 1 mL aliquot from the bottle was serial diluted from a concentration of 10^{-1} to 10^{-4}, and 1 mL of each dilution was plated onto 25 mL TSA plates in triplicate. 1 mL from the bottle was then introduced into a 100 mL sterile bag of TSB. These were incubated for 48 hours at 35˚C and formed the positive control from which the base concentration of *E. coli* initially present in the bottle was calculated. The UV light was then initiated for 5 minutes, and at intervals of 1 minute, 3 minutes, and 5 minutes the light was stopped and enough sample extracted in order to follow the same procedure as used for the positive control; namely, serial diluting 1 mL from the bottle, plating each dilution on TSA plates in triplicate, and introducing 1 mL from the bottle at each interval into a 100 mL bag of TSB. All plates and bags were incubated at 35˚C for 48 hours.

Results:
Table 1:
Log reduction and percent reduction of *E. coli* population after 1 minute of LUMA Bottle UV light treatment

Replicate	T-0 minutes	T-1 minute	Log Reduction	Percent Reduction
1	4.80E+05	1.00E+03	2.681241237	99.7917%
2	4.80E+05	2.00E+03	2.380211242	99.5833%
3	4.80E+05	1.00E+01	4.681241237	99.9979%
Average	4.80E+05	1.00E+03	3.247564572	**99.7910%**

Table 2:

Log reduction and percent reduction of *E. coli* population after 3 minutes of LUMA Bottle UV light treatment

Replicate	T-0 minutes	T-3 minutes	Log Reduction	Percent Reduction
1	4.80E+05	1.00E+03	2.681241237	99.7917%
2	4.80E+05	1.00E+01	4.681241237	99.9979%
3	4.80E+05	1.00E+01	4.681241237	99.9979%
Average	4.80E+05	3.40E+02	4.014574571	**99.9292%**

Table 3:

Log reduction and percent reduction of *E. coli* population after 5 minutes of LUMA Bottle UV light treatment

Replicate	T-0 minutes	T-5 minutes	Log Reduction	Percent Reduction
1	4.80E+05	1.00E+00	5.681241237	99.9998%
2	4.80E+05	1.00E+00	5.681241237	99.9998%
3	4.80E+05	1.00E+00	5.681241237	99.9998%
Average	4.80E+05	1.00E+00	5.681241237	**99.9998%**

Tables 1-3: The first column indicates the replicate. The second column is the initial population, as calculated by using the positive control as a base indicator. The third column shows concentration of each replicate, with each table representing a different interval. The fourth column is the log reduction after the indicated interval in the third column. The fifth column is the percent reduction of viable bacteria following use of the UV light for the interval indicated in the third column.

The 100 mL bags of TSB at each time interval showed that sufficient concentrations of viable *E. coli* remained at the 1 minute and 3 minute intervals in order to create turbidity in their respective growth-inducing media. At 5 minutes, the TSB remained clear.

All negative controls showed no growth.

Conclusion:

The results of this study demonstrate that 1 minute of continuous use effectively reduced the population of viable *E. coli* within the LUMA Bottle by 99.79%. 3 minutes reduced the viable population by 99.93%. 5 minutes yielded a 99.99% reduction. Additionally, the lack of turbidity

in the 100 mL bag at 5 minutes indicates that, at that interval, the population of viable *E. coli* sufficient to colonize a growth-inducing medium at ideal conditions had been effectively eliminated. The results of this study verify the LUMA Bottle's claims.

Sincerely,

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Joseph Holmstead
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