

# Lumin Disinfection Validation Study Design

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## Overview

This study aims to validate the Lumin disinfection cycle at cycle times of 3, 5 and 7 minutes on a challenge microbe.

## Materials

### *Biological Indicator*

To test the disinfection success of the Lumin, a biological indicator will be required. Biological indicators (BI) are characterized preparations of a specific microorganism that provides a defined and stable resistance to a specific disinfection process. Suitable biological indicators are spore-forming bacteria because they are significantly more resistant than normal microflora.

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. 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.

### *Carrier Type*

Stainless steel carriers from the Apex BI line inoculated with  $10^5$  *Geobacillus stearothermophilus*. The BI samples should be transferred to Tryptic Soy Agar when received by the test lab with dilutions of  $10^{-1}$ ,  $10^{-2}$ , and  $10^{-3}$ .

#### Preliminary Method:

1. 6mm discs inoculated with  $10^5$  *G. stearothermophilus* will be adhered to both a CPAP mask and water chamber.
2. Expose the test articles to one cycle at Lumin, with cycle times of 3 min, 5 min and 7 min.
3. Reserve 2 inoculated discs as negative controls that will not be exposed to a disinfection procedure.
4. The BI samples will then need to be transferred to Trypticase Soy Agar plates after diluting at  $10^{-4}$  (depending on inoculation population) - aim to achieve 3-30 CFU for one dilution.
5. Incubate all samples at  $35^{\circ}\text{C}$  for 48 hours and count colonies.

## Lumin Lab Study – Population Assay

Date: 11/20/17	Time: 1000
Carrier: Apex BI Line G. stearothermophilus	Technician: A. Giudice

### Description:

Following the Lumin Disinfection Validation Study Design provided, 6mm stainless steel disc carriers inoculated with the challenge strain, *G. stearothermophilus*, were adhered to both a CPAP mask and the water chamber. The test articles were labeled A1 & A2 for 3 min exposure, B1 & B2 for 5 min exposure, and C1 & C2 for 7 min exposure. Two control discs were not subjected to the Lumin cleaning cycle and labeled D1 & D2. At conclusion, carrier discs were packed in dry ice and overnighted to Microchem Laboratory.

Sample ID	Description	Lumin Cycle Duration
A1	nasal mask	3 mins
A2	water chamber	3 " "
B1	nasal mask	5 " "
B2	water chamber	5 " "
C1	nasal mask	7 " "
C2	water chamber	7 " "
D1	Control	0 " "
D2	Control	0

Performed by: A. Giudice

Date: 11/20/17



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**Client Information**

Company Name: 3B Medical

Sponsor: Alex Lucio

E-mail: alucio@3bproducts.com

**Test Information**

Test Performed: Aerobic Plate Count for *G. stearothermophilus*

Test Performed by: C. Craney

**Sample Information**

Test Set ID: 3B Medical, Inc.

Samples received: 21 NOV 2017

**Parameters**

Dilution Media: Phosphate Buffered Saline

Neutralization Media: Dey-Engley Broth (10 ml)

Plate Inc. Media: Tryptic Soy Agar

Test Samples: Inoculated Stainless Steel Carriers

Plate Inc. Temp.: 55-60°C

Incubation Period: 7 days

**Controls**

Neutralized: N/A

Growth Control: N/A

**Test Results**

Bacteria Present? See Results

Test Valid? Yes

**Notes:** Stainless steel carriers from the Apex BI line were received having been inoculated with  $\sim 10^5$  CFU *Geobacillus stearothermophilus* by the sponsor prior to shipping. Tryptic Soy Agar was enumerated with dilutions of  $10^{-1}$ ,  $10^{-2}$  and  $10^{-3}$ .

Tests Completed: 28 NOV 2017

Report Sent: 30 NOV 2017



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3B Medical, Inc. Baseline Results

Results:

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 5 Min Exposure	<5.00E+00
B2-Lumin-Dose at 5 Min Exposure	<5.00E+00
C1-Lumin-Dose at 7 Min Exposure	<5.00E+00
C2-Lumin-Dose at 7 Min Exposure	<5.00E+00
D1-CONTROL	3.10E+04
D2-CONTROL	3.10E+04

*The limit of detection for this aerobic bacteria plate count is 5 CFU/sample, values below the limit of detection are reported as <5.00E+00.*