# FINAL REPORT

# VIOGUARD KEYBOARD: LOW-LEVEL DISINFECTION EVALUATION

## 6 BULBS, 90-second Exposure

## Membrane Filtration Test

**Subject:**

Microbial disinfection test, VIOGUARD, Inc. keyboard

*Spores of Clostridium difficile*

**Document Date of Creation:**

December 11, 2017

**Date Studies Conducted:**

December 02-09, 2017

**Device Tested:** Self-Sanitizing Keyboard, Serial No UVKB-50 V2.

**Tested by:** *Cristina Romo*

**Supervisor:** *David Meller*

**Sponsor:**

VIOGUARD, INC
19201 120th AVE NE, SUITE 200
BOTHELL, WA 98011, USA

Ondine Research Laboratories, Inc
19017 120th Ave NE, Suite 102
Bothell, WA 98011
425.489.1100

# Contents

PURPOSE ..... 3
METHODS ..... 3
PROCEDURE ..... 4
Test Device Inoculation ..... 4
Disinfection Procedure ..... 5
RESULTS ..... 6
CONCLUSIONS ..... 8
APPENDIX A: Tabulated Raw Data ..... 9
APPENDIX B: Graphics ..... 15

# PURPOSE

This document reports the results of testing low-level disinfection of *Clostridium difficile* spores by the UVKB-50 V2 self-sanitizing keyboard (6 bulbs) from Vioguard, Inc. All tests were conducted within a BSL Class II facility, under non-GLP procedures (Ondine Research Laboratories Inc., Bothell, WA 98011).

# METHODS

## *Extraction Method*

A cotton swab (Puritan, Lot# 4413) saturated with peptone water (PEPT) (Criterion; Lot# 17075) was used to recover bacteria from the keyboard after the disinfection process. Vortexing and manual shaking were used to release spores from the swab into a PEPT aliquot.

## *Membrane Filtration test*

1 mL of each sampling fluid was filtered through a 0.45-µm cellulose membrane supported by a filter (Whatman; Lot# 7020610); approximately 100 mL of PEPT was passed through the filter and membrane, maintaining a horizontal orientation, to remove non-bacterial substrates. The filter membrane was then placed onto a *C. difficile*-selective agar plate (BBL; Lot# 7276544). Plates were incubated at 37°C, 80% $N_2$, 10% $CO_2$, 10% $H_2$ in an anaerobic chamber incubator for 48hours.

## *Accurate Range*

Raw plate count results for standard agar plates should be 20-200 CFU per filter, and 20 – 200 CFU per plate for spread plates. Values outside these ranges are still reported but must be considered approximate. If no colonies were found on the filters or plates, the results were reported using the *less than* symbol (<) for recovery values and the *greater than* (>) symbol for log reduction values, denoting that the recovery extent or log reduction value was outside the test detection limit.

## *Controls*

- *Environmental control*: *C. Difficile* -selective agar plate and Broth Hearth Infusion supplemented (BHIS) plates were opened to the environment on the work bench to act as an environmental control during the plating process for each test. No growth was observed on the environmental monitor plate.

- *Negative control*: A negative control media monitor (unopened agar plate), was incubated for during the process. No growth was observed on the negative media plate.

- *Diluent control*: an aliquot of PEPT was processed as a sample for each test, placed on a *C. Difficile*-selective agar plate and on a BHIS plate. All control plates were incubated at 37°C, 80% $N_2$, 10% $CO_2$, 10% $H_2$ in an anaerobic chamber incubator for 24-48 hours. No growth was observed.

- *Negative Device:* A cotton swab (Puritan; Lot# 4413) saturated with peptone water (PEPT) (Criterion; Lot# 17075) was used to recover bacteria from the keyboard (sites 1through 5) before the inoculation process. 0.1 mL was plated onto individual *C. Difficile*-selective agar plate (BBL; Lot# 7276544) using standard spreading techniques. Plates were incubated at 37°C, 80% $N_2$, 10% $CO_2$, 10% $H_2$ in an anaerobic chamber incubator for 24-48 hours. No growth was observed.

### *Variability*

Small variability in the recovery of microbes from the keyboard may be attributed to the nature of microorganisms. Different enumeration methods have the potential to exhibit different detection limits and different degrees of accuracy and reproducibility.

## PROCEDURE

### *Spores of Clostridium difficile preparation:*

*C. difficile* ATCC 17858 was plated onto BHIS and incubated at 37°C, 80% $N_2$, 10% $CO_2$, 10% $H_2$ in a chamber incubator for 24-48 hours. After incubation period, plates were scraped, and cells were resuspended in 1xPBS. Suspension was centrifuged at 3,000g for 15 min. Cells were washed 2x in 1xPBS resuspending pellet each time. Cells were incubated at 4ºC overnight to aid in lysis of vegetative and mother cells. Thereafter, cells were incubated for 20 min. at 70ºC to kill any residual vegetative cells.

### *Test Device Inoculation*

A 5% bovine serum solution was prepared using purified water (PURW) and inoculated with spores of *C. difficile* ATCC 17858, with concentration adjusted to approximately $1x10^6$ CFU/mL. A standard plate count was performed on the inoculated serum ("inoculum"), with a measured concentration of $1.15 \times 10^6$ CFU/mL.

Five sites chosen by the sponsor were inoculated on each test device (Figure 1) with 0.1 mL of the inoculum using a sterile syringe:

Figure 1: Self-Sanitizing Keyboard (6 bulbs), UVKB-50 V2, with inoculation sites.

The device was allowed to dry at ambient temperature (21 ± 2ºC) for a minimum of one hour, or until the inoculum was completely dry. Drying times are listed below (Traceable™ timer; ID# C0202):

**TABLE 1:** Drying time of the inoculum at each site

| Test | Site | Drying Time [min] |
|---|---|---|
| **Positive Control** | 1 | 59.13 |
| | 2 | 55.18 |
| | 3 | 63.08 |
| | 4 | 40.13 |
| | 5 | 49.13 |
| **Test 1** | 1 | 58.13 |
| | 2 | 69.02 |
| | 3 | 55.13 |
| | 4 | 50.13 |
| | 5 | 58.13 |
| **Test 2** | 1 | 59.13 |
| | 2 | 49.15 |
| | 3 | 49.15 |
| | 4 | 59.13 |
| | 5 | 49.15 |
| **Test 3** | 1 | 60.13 |
| | 2 | 59.10 |
| | 3 | 55.13 |
| | 4 | 58.30 |
| | 5 | 65.18 |

### *Disinfection Procedure*

Using aseptic technique, the keyboard was manually returned into the device chassis until the front door was fully closed. The power switch on the device was then turned on to initiate the disinfection cycle for **90 seconds** (Traceable™ timer; ID# C0202). After 90 seconds, the power switch was turned off and the door manually opened. The keyboard was then manually slid out of the case.

# RESULTS

## *Membrane Filtration Test*

These results are reported using the standard membrane filtration protocol. Results of the alternate protocols are also appended to this report (see Appendix A).

**TABLE 2:** DISINFECTION RESULTS. *Spores of Clostridium difficile*-SITE 1

| Test | CFU/mL | Log Reduction | Percent Reduction (%) |
|---|---|---|---|
| **1** | $2.8 \times 10^3$ | 1.8 | 98.2390 |
| **2** | $3.9 \times 10^3$ | 1.6 | 97.5472 |
| **3** | $2.8 \times 10^3$ | 1.8 | 98.2390 |

Soil Titer: $1.15 \times 10^6$ CFU/mL
Positive Device Titer: $1.59 \times 10^5$ CFU/site 1

**TABLE 3:** DISINFECTION RESULTS. *Spores of Clostridium difficile* -SITE 2

| Test | CFU/mL | Log Reduction | Percent Reduction (%) |
|---|---|---|---|
| **1** | $1.5 \times 10^3$ | 2.0 | 98.9583 |
| **2** | $2.8 \times 10^3$ | 1.7 | 98.0556 |
| **3** | $1.5 \times 10^3$ | 2.0 | 98.9583 |

Soil Titer: $1.15 \times 10^6$ CFU/mL
Positive Device Titer: $1.44 \times 10^5$ CFU/site 2

**TABLE 4**: DISINFECTION RESULTS. *Spores of Clostridium difficile* -SITE 3

| Test | CFU/mL | Log Reduction | Percent Reduction (%) |
|---|---|---|---|
| **1** | $1.8 \times 10^3$ | 2.0 | 99.0000 |
| **2** | $2.5 \times 10^3$ | 1.9 | 98.6111 |
| **3** | $2.0 \times 10^3$ | 2.0 | 98.8889 |

Soil Titer: $1.15 \times 10^6$ CFU/mL
Positive Device Titer: $1.80 \times 10^5$ CFU/site 3

**TABLE 5:** DISINFECTION RESULTS. *Spores of Clostridium difficile* -SITE 4

| Test | CFU/mL | Log Reduction | Percent Reduction (%) |
|---|---|---|---|
| **1** | $2.3 \times 10^3$ | 1.7 | 98.0833 |
| **2** | $4.0 \times 10^3$ | 1.5 | 96.6667 |
| **3** | $1.8 \times 10^3$ | 1.8 | 98.5000 |

Soil Titer: $1.15 \times 10^6$ CFU/mL
Positive Device Titer: $1.20 \times 10^5$ CFU/site 4

**TABLE 6:** DISINFECTION RESULTS. *Spores of Clostridium difficile* -SITE 5

| Test | CFU/mL | Log Reduction | Percent Reduction (%) |
|---|---|---|---|
| **1** | $2.2 \times 10^3$ | 1.7 | 98.1666 |
| **2** | $2.5 \times 10^3$ | 1.7 | 97.9167 |
| **3** | $1.7 \times 10^3$ | 1.8 | 98.5833 |

Soil Titer: $1.15 \times 10^6$ CFU/mL
Positive Device Titer: $1.03 \times 10^5$ CFU/site 5

## CONCLUSIONS

Interpretation of the data is the responsibility of the study sponsor.

| APPROVALS | | |
|---|---|---|
| Engineering Manager | David Meller | |
| Laboratory Manager | Cristina Romo | |

# APPENDIX A:

# Tabulated Raw Data

# MEMBRANE FILTRATION DATA

Positive control, **Site 1**

Organism: spores of *Clostridium difficile* ATCC 17858

Number of Positives: 1

**POSITIVE CONTROL, TITER CALCULATION**

| Positive Device | Volume IN (mL) | Diluent Plated (mL) | CFU/plate | CFU/mL |
|---|---|---|---|---|
| **1** | 100.0 | 0.1 | 159 | $1.59 \times 10^5$ |
| | | Positive Control Titer: | | $1.59 \times 10^5$ |
| | | Log 10 | | 5.2 |

**Site 1**

Organism: spores of *Clostridium difficile* ATCC 17858

Number of devices tested: 1

Number of replicate tests: 3

**SITE 1 after 90-sec disinfection cycle**

| Test number | Volume IN (mL) | CFU/plate | CFU/mL | Log Reduction | Percent Reduction (%) |
|---|---|---|---|---|---|
| **1** | 100.0 | 28 | $2.8 \times 10^3$ | 1.8 | 98.2390 |
| **2** | 100.0 | 39 | $3.9 \times 10^3$ | 1.6 | 97.5472 |
| **3** | 100.0 | 28 | $2.8 \times 10^3$ | 1.8 | 98.2390 |

CFU= Colony forming unit
< = None detected

| **Inoculum** | |
|---|---|
| CFU Plate 1: | 117 |
| CFU Plate 2: | 128 |
| CFU Plate 3: | 101 |
| Dilution: | 1000 |
| Volume plated (ml): | 0.1 |
| Initial CFU/mL: | $1.15 \times 10^6$ CFU/mL |

Positive control, **Site 2**

Organism: spores of *Clostridium difficile* ATCC 17858

Number of Positives: 1

**POSITIVE CONTROL, TITER CALCULATION**

| Positive Device | Volume IN (mL) | Diluent Plated (mL) | CFU/plate | CFU/mL |
|---|---|---|---|---|
| **1** | 100.2 | 0.1 | 144 | $1.44 \times 10^5$ |
| | | **Positive Control Titer:** | | $1.44 \times 10^5$ |
| | | **Log 10** | | 5.2 |

**Site 2**

Organism: spores of *Clostridium difficile* ATCC 17858

Number of devices tested: 1

Number of replicate tests: 3

**SITE 2 after 90-sec disinfection cycle**

| Test number | Volume IN (mL) | CFU/plate | CFU/mL | Log Reduction | Percent Reduction (%) |
|---|---|---|---|---|---|
| **1** | 100.1 | 15 | $1.5 \times 10^3$ | 2.0 | 98.9583 |
| **2** | 100.4 | 28 | $2.8 \times 10^3$ | 1.7 | 98.0556 |
| **3** | 100.1 | 15 | $1.5 \times 10^3$ | 2.0 | 98.9583 |

CFU= Colony forming unit
< = None detected

| Inoculum | |
|---|---|
| CFU Plate 1: | 117 |
| CFU Plate 2: | 128 |
| CFU Plate 3: | 101 |
| Dilution: | 1000 |
| Volume plated (ml): | 0.1 |
| Initial CFU/mL: | $1.15 \times 10^6$ CFU/mL |

Positive control, **Site 3**

Organism: spores of *Clostridium difficile* ATCC 17858

Number of Positives: 1

**POSITIVE CONTROL, TITER CALCULATION**

| Positive Device | Volume IN (mL) | Diluent Plated (mL) | CFU/plate | CFU/mL |
|---|---|---|---|---|
| **1** | 100.0 | 0.1 | 180 | $1.80 \times 10^5$ |
| | | Positive Control Titer: | | $1.80 \times 10^5$ |
| | | Log 10 | | 5.3 |

**Site 3**

Organism: spores of *Clostridium difficile* ATCC 17858

Number of devices tested: 1

Number of replicate tests: 3

**SITE 3 after 90-sec disinfection cycle**

| Device | Volume IN (mL) | CFU/plate | CFU/mL | Log Reduction | Percent Reduction (%) |
|---|---|---|---|---|---|
| **1** | 100.0 | 18 | $1.8 \times 10^3$ | 2.0 | 99.0000 |
| **2** | 100.1 | 25 | $2.5 \times 10^3$ | 1.9 | 98.6111 |
| **3** | 100.2 | 20 | $2.0 \times 10^3$ | 2.0 | 98.8889 |

CFU= Colony forming unit
< = None detected

| Inoculum | |
|---|---|
| CFU Plate 1: | 117 |
| CFU Plate 2: | 128 |
| CFU Plate 3: | 101 |
| Dilution: | 1000 |
| Volume plated (ml): | 0.1 |
| Initial CFU/mL: | $1.15 \times 10^6$ CFU/mL |

Positive control, **Site 4**

Organism: spores of *Clostridium difficile* ATCC 17858

Number of Positives: 1

**POSITIVE CONTROL, TITER CALCULATION**

| Positive Device | Volume IN (mL) | Diluent Plated (mL) | CFU/plate | CFU/mL |
|---|---|---|---|---|
| **1** | 100.1 | 0.1 | 120 | $1.20 \times 10^5$ |
| | | **Positive Control Titer:** | | $1.20 \times 10^5$ |
| | | **Log 10** | | 5.1 |

**Site 4**

Organism: spores of *Clostridium difficile* ATCC 17858

Number of devices tested: 1

Number of replicate tests: 3

**SITE 4 after 90-sec disinfection cycle**

| Test number | Volume IN (mL) | CFU/plate | CFU/mL | Log Reduction | Percent Reduction (%) |
|---|---|---|---|---|---|
| **1** | 100.1 | 23 | $2.3 \times 10^3$ | 1.7 | 98.0833 |
| **2** | 100.5 | 40 | $4.0 \times 10^3$ | 1.5 | 96.6667 |
| **3** | 100.0 | 18 | $1.8 \times 10^3$ | 1.8 | 98.5000 |

CFU= Colony forming unit
< = None detected

| Inoculum | |
|---|---|
| CFU Plate 1: | 117 |
| CFU Plate 2: | 128 |
| CFU Plate 3: | 101 |
| Dilution: | 1000 |
| Volume plated (ml): | 0.1 |
| Initial CFU/mL: | $1.15 \times 10^6$ CFU/mL |

Positive control, **Site 5**

Organism: spores of *Clostridium difficile* ATCC 17858

Number of Positives: 1

**POSITIVE CONTROL, TITER CALCULATION**

| Positive Device | Volume IN (mL) | Diluent Plated (mL) | CFU/plate | CFU/mL |
|---|---|---|---|---|
| **1** | 100.5 | 0.1 | 103 | $1.03 \times 10^5$ |
| | | **Positive Control Titer:** | | $1.03 \times 10^5$ |
| | | **Log 10** | | 5.0 |

**Site 5**

Organism: spores of *Clostridium difficile* ATCC 17858

Number of devices tested: 1

Number of replicate tests: 3

**SITE 5 after 90-sec disinfection cycle**

| Test number | Volume IN (mL) | CFU/plate | CFU/mL | Log Reduction | Percent Reduction (%) |
|---|---|---|---|---|---|
| **1** | 100.2 | 22 | $2.2 \times 10^3$ | 1.7 | 98.1666 |
| **2** | 100.0 | 25 | $2.5 \times 10^3$ | 1.7 | 97.9167 |
| **3** | 100.0 | 17 | $1.7 \times 10^3$ | 1.8 | 98.5833 |

CFU= Colony forming unit
< = None detected

| Inoculum | |
|---|---|
| CFU Plate 1: | 117 |
| CFU Plate 2: | 128 |
| CFU Plate 3: | 101 |
| Dilution: | 1000 |
| Volume plated (ml): | 0.1 |
| Initial CFU/mL: | $1.15 \times 10^6$ CFU/mL |

# APPENDIX B:

# Summary Figures

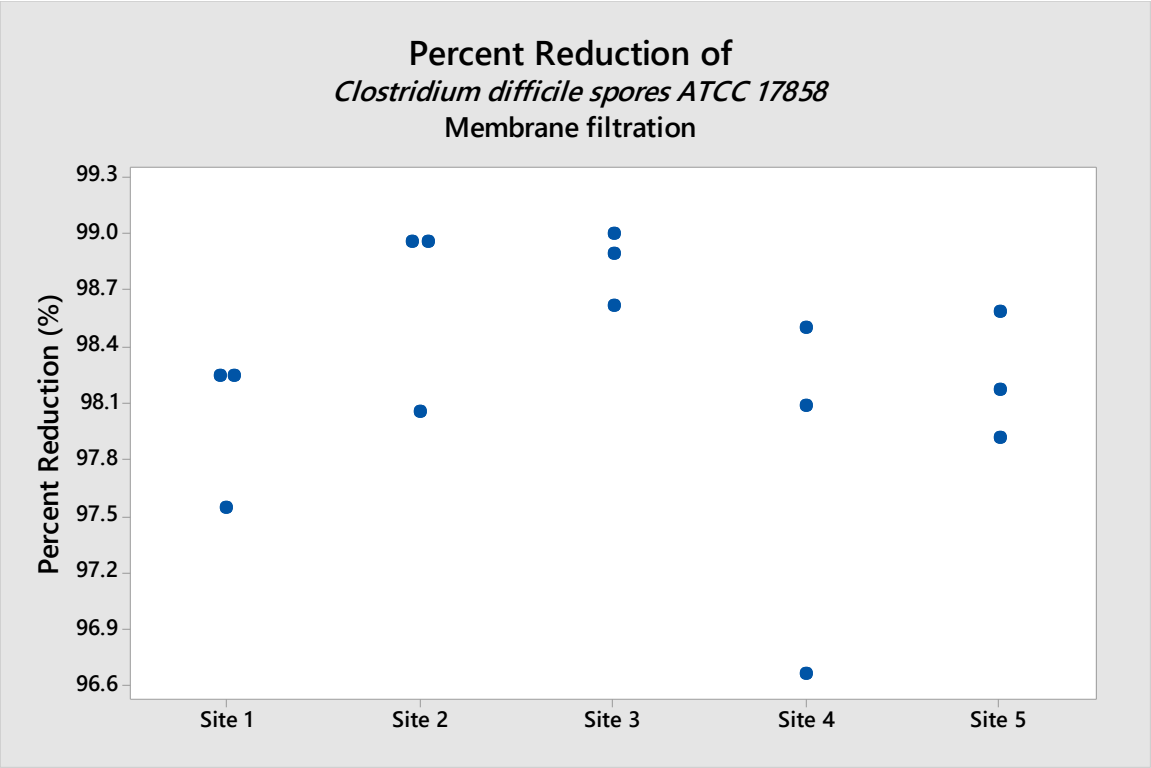


**Figure 1:** Percent reduction of *Clostridium difficile* ATCC 217858. Low-level disinfection evaluation with membrane filtration test.

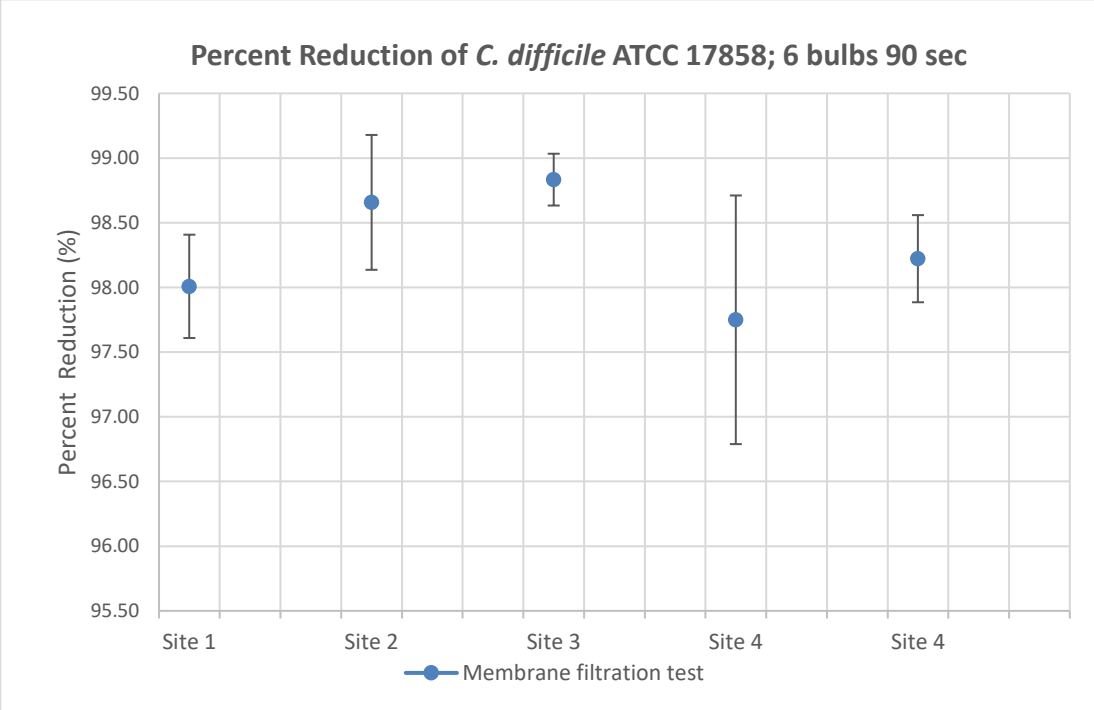


**Figure 3:** Percent reduction of *Clostridium difficile* ATCC 217858 after 90 seconds

# End of document