FINAL REPORT

LOW LEVEL DISINFECTION EVALUATION – VIOGUARD KEYBOARD

Pseudomonas aeruginosa

Subject:

Microbial disinfection test, VIOGUARD, Inc keyboard

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

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Sponsor:

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PURPOSE

The purpose of this document is to report the test results of low-level disinfection of *Pseudomonas aeruginosa* by the UVKB-50 V2 self-sanitizing keyboard from Vioguard, Inc. All tests were conducted within a BSL Class II facility, non-GLP (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 cells from the swab into a PEPT aliquot

Microbial Enumeration test:

- *Membrane Filtration test*: 1 to 10 mL of each sampling fluid was filtered through a 0.45-µm cellulose membrane supported by a filter (Pall Lot #70531B); 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 mannitol salt agar plate (Cetrimide agar, Criterion Lot# 14190). Plates were incubated at 37°C, 5% CO_2 in an Air Jacket Incubator (NUAIRE Model NU-425-400) for 24-48 hours

- *Spread Plate test*: 10- fold dilution was performed for each sample and then 0.1 mL of each dilution was plated onto individual Cetrimide agar (Criterion Lot# 14190) plates using standard spreading techniques. Plates were incubated at 37°C, 5% CO_2 in an Air Jacket Incubator (NUAIRE Model NU-425-400) for 24-48 hours

- *Direct Plating test*: 10- fold dilution was performed for each sample and then 20 µL of solution was plated onto individual Cetrimide agar (Criterion Lot# 14190) plates. Plates were incubated at 37°C, 5% CO_2 in an Air Jacket Incubator (NUAIRE Model NU-425-400) for 24-48 hours.

Accurate range:

The raw plate count results for standard agar plates should be within 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 are found on the filters or on the plates, the results will be reported using the *less than* symbol (<) for recovery values and the *greater than* (>) symbol for log reduction values. This denotes the recovery extent, or log reduction value, is outside the test detection limit

Controls:

- *Environmental control*: A cetrimide agar plate was 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. No growth was observed on the negative media monitor plate at any time.
- *Negative control*: A negative control media monitor (unopened agar plate), was also incubated for each agar type during the process. No growth was observed on the negative media monitor plate at any time.
- *Diluent control*: an aliquot of PEPT was processed as a sample for each test, placed on a cetrimide agar plate. All control plates were incubated at at 37°C, 5% CO_2 in an Air Jacket

Incubator (NUAIRE Model NU-425-400) for 24-48 hours. No growth was observed on the negative media monitor plate at any time.

- *Negative Device:* A cotton swab (Puritan, Lot# 4413) saturated with peptone water (PEPT) (Criterion LOT #17075) was used to recover bacteria present from the keyboard (sites 1, 2, 3 and 4) before the inoculation process. 0.1 mL was plated onto Cetrimide agar (Criterion Lot# 14190) and TSA plates using standard spreading techniques. Plates were incubated at 37°C, 5% CO_2 in an Air Jacket Incubator (NUAIRE Model NU-425-400) for 24-48 hour. No growth was observed on the negative media monitor plate at any time.

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.

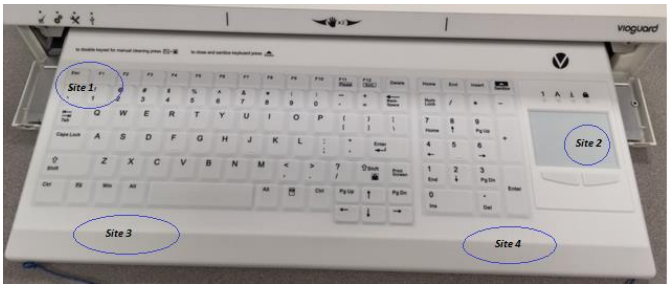
PROCEDURE

Test Device Inoculation

A 5% bovine serum solution was prepared using purified water (PURW) and inoculated with *Pseudomonas aeruginosa* ATCC 9027 (*P. aeruginosa*), with concentration adjusted to ~1.0x 10^7 CFU/mL via spectrophotometric absorption in a 1-cm cuvette at 420 nm (Spectrophotometer Genesys 10s). A standard plate count was performed on the inoculated serum ("inoculum"), with a concentration detected of 2.23 x 10^7 CFU/mL, validating the spectrophotometric test.

Four 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, UVKB-50 V2. 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	44.32
	2	42.24
	3	43.12
	4	45.18
Test 1	1	47.38
	2	56.59
	3	55.02
	4	51.13
Test 2	1	46.43
	2	42.13
	3	49.13
	4	53.02
Test 3	1	47.13
	2	49.52
	3	44.25
	4	51.13

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). The electrical power consumed by the device was measured with a P4400 KILL A WATT™ unit (see Table 2). After 90 seconds, the power switch was turned off and the door manually opened. The keyboard was then manually slid out of the case.

TABLE 2: Measured power during the disinfection cycle

Device	Power [W]
Positive Control	N/A
Test 1	Min: 44.2 Max: 46.1
Test 2	Min: 44.0 Max: 46.4
Test 3	Min: 44.0* Max: 45.7

*In the last 5 seconds, the 43.7 – 43.9 W were registered.

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 3: DISINFECTION RESULTS. *Pseudomonas aeruginosa* ATCC 9027 -SITE 1

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	<1 x 10^0	> 4.60	> 99.9975
2	<1 x 10^0	> 4.60	> 99.9975
3	<1 x 10^0	> 4.60	> 99.9975

Soil Titer: 4.58 x 10^7 CFU/mL
Positive Device Titer: 3.96 x 10^4 CFU/site 1

TABLE 3: DISINFECTION RESULTS. *Pseudomonas aeruginosa* ATCC 9027 -SITE 2

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	<1 x 10^0	> 4.70	> 99.9980
2	<1 x 10^0	> 4.70	> 99.9980
3	<1 x 10^0	> 4.70	> 99.9980

Soil Titer: 4.58 x 10^7 CFU/mL
Positive Device Titer: 4.97 x 10^4 CFU/site 2

TABLE 4: DISINFECTION RESULTS. *Pseudomonas aeruginosa* ATCC 9027 -SITE 3

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	<1 x 10^0	> 4.78	> 99.9983
2	<1 x 10^0	> 4.78	> 99.9983
3	<1 x 10^0	> 4.78	> 99.9983

Soil Titer: 4.58 x 10^7 CFU/mL
Positive Device Titer: 6.03 x 10^4 CFU/site 3

TABLE 5: DISINFECTION RESULTS. *Pseudomonas aeruginosa* ATCC 9027 -SITE 4

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	<1 x 10^0	> 4.82	> 99.9985
2	<1 x 10^0	> 4.82	> 99.9985
3	<1 x 10^0	> 4.82	> 99.9985

Soil Titer: 4.58 x 10^7 CFU/mL
Positive Device Titer: 6.67 x 10^4 CFU/site 4

Spread Plate Test

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

TABLE 3: DISINFECTION RESULTS. *Pseudomonas aeruginosa* ATCC 9027 -SITE 1

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	$<1 \times 10^0$	> 6.18	> 99.9999
2	$<1 \times 10^0$	> 6.18	> 99.9999
3	$<1 \times 10^0$	> 6.18	> 99.9999

Soil Titer: 4.58×10^7 CFU/mL
Positive Device Titer: 1.52×10^6 CFU/site 1

TABLE 3: DISINFECTION RESULTS. *Pseudomonas aeruginosa* ATCC 9027 -SITE 2

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	$<1 \times 10^0$	> 5.30	> 99.9995
2	$<1 \times 10^0$	> 5.30	> 99.9995
3	$<1 \times 10^0$	> 5.30	> 99.9995

Soil Titer: 4.58×10^7 CFU/mL
Positive Device Titer: 2.01×10^6 CFU/site 2

TABLE 4: DISINFECTION RESULTS. *Pseudomonas aeruginosa* ATCC 9027 -SITE 3

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	$<1 \times 10^0$	> 5.11	> 99.9992
2	$<1 \times 10^0$	> 5.11	> 99.9992
3	$<1 \times 10^0$	> 5.11	> 99.9992

Soil Titer: 4.58×10^7 CFU/mL
Positive Device Titer: 1.29×10^5 CFU/site 3

TABLE 5: DISINFECTION RESULTS. *Pseudomonas aeruginosa* ATCC 9027 -SITE 4

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	$<1 \times 10^0$	> 4.99	> 99.9990
2	$<1 \times 10^0$	> 4.99	> 99.9990
3	$<1 \times 10^0$	> 4.99	> 99.9990

Soil Titer: 4.58×10^7 CFU/mL
Positive Device Titer: 9.8×10^4 CFU/site 4

CONCLUSIONS

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

APPROVALS		
Director	David Meller	
Laboratory Manager	Cristina Romo	

APPENDIX A: Tabulated Raw Data

MEMBRANE FILTRATION DATA

Positive control, **Site 1**

Organism: *Pseudomonas aeruginosa* ATCC 9027

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Volume IN (mL)	Diluent Plated (mL)	CFU/plate	CFU/mL
1	101.6	0.1	43	4.4×10^4
2	101.6	0.1	38	3.8×10^4
3	101.6	0.1	37	3.7×10^4
		Positive Control Titer:		3.96×10^4
		Log 10		4.60

Site 1

Organism: *Pseudomonas aeruginosa* ATCC 9027

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	104.0	<1	$<1 \times 10^0$	> 4.60	> 99.9975
2	102.7	<1	$<1 \times 10^0$	> 4.60	> 99.9975
3	102.4	<1	$<1 \times 10^0$	> 4.60	> 99.9975

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	87
CFU Plate 2:	97
CFU Plate 3:	93
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	4.58×10^7

Positive control, **Site 2**

Organism: *Pseudomonas aeruginosa* ATCC 9027

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Volume IN (mL)	Diluent Plated (mL)	CFU/plate	CFU/mL
1	101.3	0.1	42	4.2×10^4
2	101.3	0.1	55	5.5×10^4
3	101.3	0.1	52	5.2×10^4
		Positive Control Titer:		4.97×10^4
		Log 10		4.70

Site 2

Organism: *Pseudomonas aeruginosa* ATCC 9027

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	103.8	<1	$<1 \times 10^0$	>4.70	> 99.9980
2	101.0	<1	$<1 \times 10^0$	>4.70	> 99.9980
3	100.3	<1	$<1 \times 10^0$	>4.70	> 99.9980

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	87
CFU Plate 2:	97
CFU Plate 3:	93
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	4.58×10^7

Positive control, **Site 3**

Organism: *Pseudomonas aeruginosa* ATCC 9027

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Volume IN (mL)	Diluent Plated (mL)	CFU/plate	CFU/mL
1	102.3	0.1	88	8.8×10^4
2	102.3	0.1	45	4.5×10^4
3	102.3	0.1	48	4.8×10^4
		Positive Control Titer:		6.03×10^4
		Log 10		4.78

Site 3

Organism: *Pseudomonas aeruginosa* ATCC 9027

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	99.8	<1	$<1 \times 10^0$	> 4.78	> 99.9983
2	100.2	<1	$<1 \times 10^0$	> 4.78	> 99.9983
3	100.4	<1	$<1 \times 10^0$	> 4.78	> 99.9983

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	87
CFU Plate 2:	97
CFU Plate 3:	93
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	4.58×10^7

Positive control, **Site 4**

Organism: *Pseudomonas aeruginosa* ATCC 9027

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Volume IN (mL)	Diluent Plated (mL)	CFU/plate	CFU/mL
1	101.1	0.1	89	8.9×10^4
2	101.1	0.1	52	5.2×10^4
3	101.1	0.1	59	5.9×10^4
		Positive Control Titer:		6.67×10^4
		Log 10		4.82

Site 4

Organism: *Pseudomonas aeruginosa* ATCC 9027

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	104.7	<1	$<1 \times 10^0$	> 4.82	99.9985
2	101.4	<1	$<1 \times 10^0$	> 4.82	99.9985
3	101.2	<1	$<1 \times 10^0$	> 4.82	99.9985

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	87
CFU Plate 2:	97
CFU Plate 3:	93
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	4.58×10^7

SPREAD PLATING DATA

Positive control, **Site 1**

Organism: *Pseudomonas aeruginosa* ATCC 9027

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Volume IN (mL)	Dilution factor	CFU/plate	CFU/mL
1	0.1	1:1000	155	1.55×10^6
2	0.1	1:1000	148	1.48×10^6
3	0.1	1:1000	152	1.52×10^6
		Positive Control Titer:		1.52×10^6
		Log 10		6.18

Site 1

Organism: *Pseudomonas aeruginosa* ATCC 9027

Number of devices tested: 1

Number of replicate tests: 3

SITE 1 after 90-sec disinfection cycle

Device	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL	Log Reduction	Percent Reduction (%)
1	0.1	10^0	<1	$<1 \times 10^0$	>6.18	> 99.9999
2	0.1	10^0	<1	$<1 \times 10^0$	>6.18	> 99.9999
3	0.1	10^0	<1	$<1 \times 10^0$	>6.18	> 99.9999

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	87
CFU Plate 2:	97
CFU Plate 3:	93
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	4.58×10^7

Positive control, **Site 2**

Organism: *Pseudomonas aeruginosa* ATCC 9027

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL
1	0.1	1:100	210	2.1×10^6
2	0.1	1:100	200	2.0×10^6
3	0.1	1:100	193	1.9×10^6
		Positive Control Titer:		2.01×10^5
		Log 10		5.30

Site 2

Organism: *Pseudomonas aeruginosa* ATCC 9027

Number of devices tested: 1

Number of replicate tests: 3

SITE 2 after 90-sec disinfection cycle

Device	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL	Log Reduction	Percent Reduction (%)
1	0.1	10^0	<1	$<1 \times 10^0$	>5.30	99.9995
2	0.1	10^0	<1	$<1 \times 10^0$	>5.30	99.9995
3	0.1	10^0	<1	$<1 \times 10^0$	>5.30	99.9995

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	87
CFU Plate 2:	97
CFU Plate 3:	93
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	4.58×10^7

Positive control, **Site 3**

Organism: *Pseudomonas aeruginosa* ATCC 9027

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL
1	0.1	1:100	121	1.21×10^5
2	0.1	1:100	138	1.38×10^5
3	0.1	1:100	127	1.27×10^5
		Positive Control Titer:		1.29×10^5
		Log 10		5.11

Site 3

Organism: *Pseudomonas aeruginosa* ATCC 9027

Number of devices tested: 1

Number of replicate tests: 3

SITE 3 after 90-sec disinfection cycle

Device	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL	Log Reduction	Percent Reduction (%)
1	0.1	10^0	<1	$<1 \times 10^0$	>5.11	99.9992
2	0.1	10^0	<1	$<1 \times 10^0$	>5.11	99.9992
3	0.1	10^0	<1	$<1 \times 10^0$	>5.11	99.9992

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	87
CFU Plate 2:	97
CFU Plate 3:	93
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	4.58×10^7

Positive control, **Site 4**

Organism: *Pseudomonas aeruginosa* ATCC 9027

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL
1	0.1	1:100	102	1.02×10^5
2	0.1	1:100	98	9.8×10^4
3	0.1	1:100	95	9.5×10^4
		Positive Control Titer:		9.8×10^4
		Log 10		4.99

Site 4

Organism: *Pseudomonas aeruginosa* ATCC 9027

Number of devices tested: 1

Number of replicate tests: 3

SITE 3 after 90 sec-disinfection cycle

Device	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL	Log Reduction	Percent Reduction (%)
1	0.1	10^0	<1	$<1 \times 10^0$	>4.99	99.9990
2	0.1	10^0	<1	$<1 \times 10^0$	>4.99	99.9990
3	0.1	10^0	<1	$<1 \times 10^0$	>4.99	99.9990

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	87
CFU Plate 2:	97
CFU Plate 3:	93
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	4.58×10^7

DIRECT PLATING DATA

Positive control, **Site 1**

Organism: *Pseudomonas aeruginosa* ATCC 9027

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL
1	0.02	1:1000	51	2.55×10^6
2	0.02	1:1000	48	2.40×10^6
3	0.02	1:1000	55	2.75×10^6
		Positive Control Titer:		2.57×10^6
		Log 10		6.41

Site 1

Organism: *Pseudomonas aeruginosa* ATCC 9027

Number of devices tested: 1

Number of replicate tests: 3

SITE 1 after 90-sec disinfection cycle

Device	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL	Log Reduction	Percent Reduction (%)
1	0.02	10^0	<1	$<1.0 \times 10^0$	> 6.41	> 99.9999
2	0.02	10^0	<1	$<1.0 \times 10^0$	> 6.41	>99.9999
3	0.02	10^0	<1	$<1.0 \times 10^0$	> 6.41	>99.9999

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	87
CFU Plate 2:	97
CFU Plate 3:	93
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	4.58×10^7

Positive control, **Site 2**

Organism: *Pseudomonas aeruginosa* ATCC 9027

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL
1	0.02	1:1000	38	1.90×10^6
2	0.02	1:1000	43	2.15×10^6
3	0.02	1:1000	45	2.25×10^6
		Positive Control Titer:		2.1×10^6
		Log 10		6.32

Site 2

Organism: *Pseudomonas aeruginosa* ATCC 9027

Number of devices tested: 1

Number of replicate tests: 3

SITE 2 after 90-sec disinfection cycle

Device	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL	Log Reduction	Percent Reduction (%)
1	0.02	10^0	<1	$<1.0 \times 10^0$	> 6.32	>99.9999
2	0.02	10^0	<1	$<1.0 \times 10^0$	> 6.32	>99.9999
3	0.02	10^0	<1	$<1.0 \times 10^0$	> 6.32	>99.9999

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	87
CFU Plate 2:	97
CFU Plate 3:	93
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	4.58×10^7

Positive control, **Site 3**

Organism: *Pseudomonas aeruginosa* ATCC 9027

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL
1	0.02	1:1000	89	4.45×10^6
2	0.02	1:1000	93	4.65×10^6
3	0.02	1:10000	15	7.50×10^6
		Positive Control Titer:		5.53×10^6
		Log 10		6.74

Site 3

Organism: *Pseudomonas aeruginosa* ATCC 9027

Number of devices tested: 1

Number of replicate tests: 3

SITE 3 after 90-sec disinfection cycle

Device	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL	Log Reduction	Percent Reduction (%)
1	0.02	10^0	<1	$<1.0 \times 10^0$	>6.74	>99.9999
2	0.02	10^0	<1	$<1.0 \times 10^0$	>6.74	>99.9999
3	0.02	10^0	<1	$<1.0 \times 10^0$	>6.74	>99.9999

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	87
CFU Plate 2:	97
CFU Plate 3:	93
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	4.58×10^7

Positive control, **Site 4**

Organism: *Pseudomonas aeruginosa* ATCC 9027

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL
1	0.02	1:1000	36	1.80×10^6
2	0.02	1:1000	59	2.95×10^6
3	0.02	1:1000	42	2.10×10^6
		Positive Control Titer:		2.28×10^6
		Log 10		6.36

Site 4

Organism: *Pseudomonas aeruginosa* ATCC 9027

Number of devices tested: 1

Number of replicate tests: 3

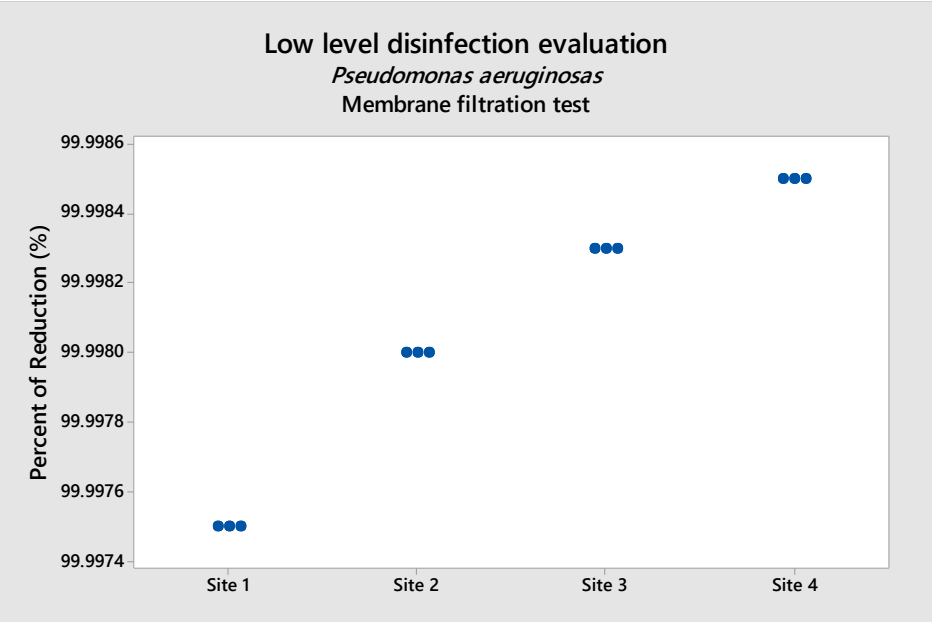
SITE 3 after 90-sec disinfection cycle

Device	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL	Log Reduction	Percent Reduction (%)
1	0.02	10^0	<1	$<1.0 \times 10^0$	>6.36	>99.9999
2	0.02	10^0	<1	$<1.0 \times 10^0$	>6.36	>99.9999
3	0.02	10^0	<1	$<1.0 \times 10^0$	>6.36	>99.9999

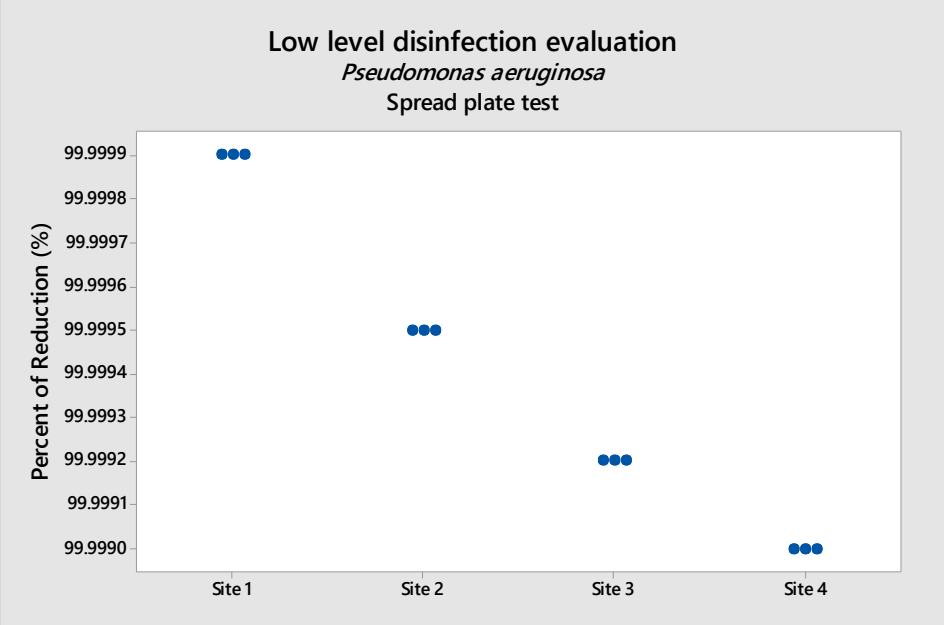
CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	87
CFU Plate 2:	97
CFU Plate 3:	93
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	4.58×10^7

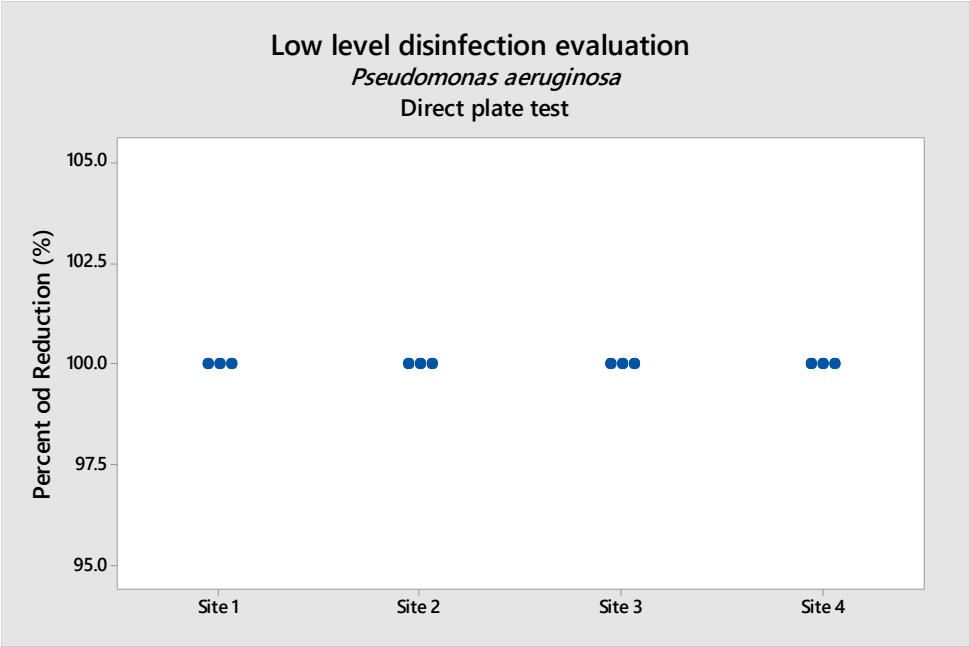
APPENDIX B: Graphics



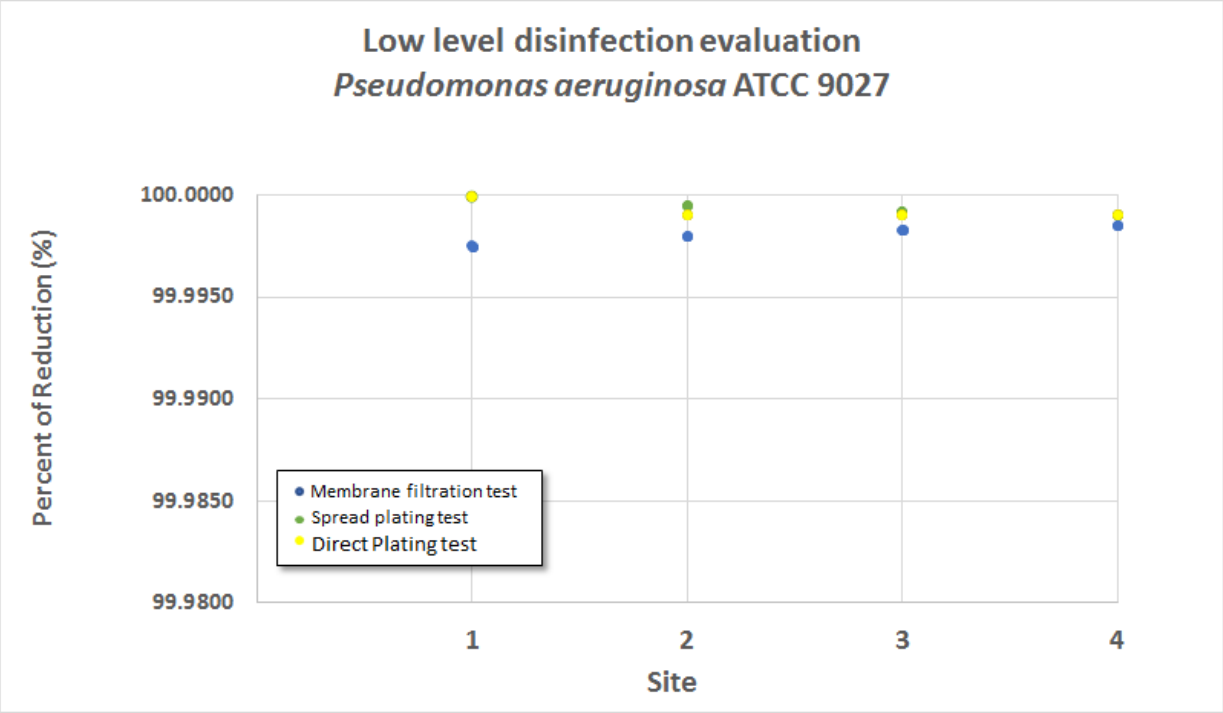
Graphic 1: Percent of reduction of low level disinfection evaluation of *Pseudomonas aeruginosa* ATCC 9027. Membrane filtration test.



Graphic 2: Percent of reduction of low level disinfection evaluation of *Pseudomonas aeruginosa* ATCC 9027. Spread Plating test.



Graphic 3: Percent of reduction of low level disinfection evaluation of *Pseudomonas aeruginosa* ATCC 9027. Direct Plating.



Graphic 4: Percent of reduction of low level disinfection evaluation of *Pseudomonas aeruginosa* ATCC 9027.

APPENDIX C: Raw Data

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LOW LEVEL DISINFECTION EVALUATION VALIDATION FORMS

Date: _____

Staphylococcus aureus ATCC 25923				
10^{-3}	10^{-4}	10^{-5}	CFU/mL	Average

CONTROL RESULTS:
MEDIA _____
MEDIA CONTROL _____ CFU/mL
ENVIRONMENTAL PLATE: _____ CFU DETECTED
DEVICE NEGATIVE CONTROL:
Site 1 _____ CFU/mL Site 2 _____ CFU/mL
Site 3 _____ CFU/mL Site 4 _____ CFU/mL
INCUBATOR: _____ TEMP _____ °C

Date: 05/23/2017

Pseudomonas aeruginosa ATCC 9077				
10^{-3}	10^{-4}	10^{-5}	CFU/mL	Average
>	87	19	4.3×10^{7}	4.58×10^{7}
>	97	26	4.8×10^{7}	
>	93	22	4.65×10^{7}	

CONTROL RESULTS:
MEDIA Cetrimide
MEDIA CONTROL 0 CFU/mL
ENVIRONMENTAL PLATE: 0 CFU DETECTED
DEVICE NEGATIVE CONTROL:
Site 1 0 CFU/mL Site 2 0 CFU/mL
Site 3 0 CFU/mL Site 4 0 CFU/mL
INCUBATOR: 0336 TEMP 36.6 °C

Date: _____

Escherichia coli ATCC 25922				
10^{-3}	10^{-4}	10^{-5}	CFU/mL	Average

CONTROL RESULTS:
MEDIA _____
MEDIA CONTROL _____ CFU/mL
ENVIRONMENTAL PLATE: _____ CFU DETECTED
DEVICE NEGATIVE CONTROL:
Site 1 _____ CFU/mL Site 2 _____ CFU/mL
Site 3 _____ CFU/mL Site 4 _____ CFU/mL
INCUBATOR: _____ TEMP _____ °C

Date: _____

Klebsiella pneumoniae ATCC 13883				
10^{-3}	10^{-4}	10^{-5}	CFU/mL	Average

CONTROL RESULTS:
MEDIA _____
MEDIA CONTROL _____ CFU/mL
ENVIRONMENTAL PLATE: _____ CFU DETECTED
DEVICE NEGATIVE CONTROL:
Site 1 _____ CFU/mL Site 2 _____ CFU/mL
Site 3 _____ CFU/mL Site 4 _____ CFU/mL
INCUBATOR: _____ TEMP _____ °C

EXTRACTION METHOD:

IMMERSION:

ENUMERATION:

- [x] MEMBRANE FILTRATION
- [x] DIRECT PLATING
- [x] SPREAD PLATE: AMOUNT PLATED 0.1mL

MEDIA

- [x] TSA
- [ ] TSB
- [ ] MSA
- [ ] EMB
- [ ] MAC CONKEY
- [x] CETRAMIDE
- [ ] MUELLER HINTON
- [ ] OTHER

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LOW LEVEL DISINFECTION EVALUATION VALIDATION FORMS

Date: 21/May/17 – 22/May/17

Organism(s)	P. aeruginosa
ATCC #	9027
Culture Age	18 hours
Plate Media/Lot#	TSA ON LOT # 051817
Incubator ID#	0336
Temperature:	36 ± 2°C

Time/Date In:	05/21/17	5:00 p.m
Time/Date Out:	05/22/17	11:05 a.m

Inoculum preparation	
Abs at 420 nm	0.158
Prepared in/Lot #	5% Bovine Serum ON LOT # 052217
Spectrophotometer ID#	C0216 Thermo Scientific Genesys 10S

Equipment:

	Ondine ID#	Calibration verified	Additional equipment ☐ N/A
Micropipettes	C0022, C0034, 0388, C0030	☑ Yes ☐ N/A	
Vortex	C0075 ; C0073	☐ Yes ☑ N/A	
Timer	C0202	☑ Yes ☐ N/A	
Biosafety Cabinet/Hood	0338	☑ Yes ☐ N/A	
Balance	0350 ; C0066	☑ Yes ☐ N/A	
Thermometer	C0203	☑ Yes ☐ N/A	
Aspirator pipet	C0072	☐ Yes ☑ N/A	
Colony counter	S/N	☐ Yes ☑ N/A	

Supplies	**LOT #**	**EXP /Manufacturer**	**Additional equipment ☐ N/A**
Peptone-Tween water	17075 ON LOT # 051917	Criterion Jan'20	
Purified water	1610H29	Ricca Oct-2017	
Deionized water	VE 060410	Integra	
Bovine Serum	Cat supplement w/ iron M1815	Seradigm Jun'20	
Sterile swabs	4413	Puritan 10/20	
Filter	7053135	Pall	
Filter unit	N/A	Nalgene	
PBS	LOT ON # 030617		
TSB	5117514	BD 03/20	
Tween 80	CH2B	Integra 208-08	

Sample contamination

Number of devices contaminated: 1

Number of test: 4

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LOW LEVEL DISINFECTION EVALUATION VALIDATION FORMS

Sample contamination

P. aeruginosa ATCC 9027
05/22/2017

☑ On each test device, inoculate the following test sites with the same bacteria:

Site of inoculation	Amount inoculated
Site #1: Esc, F1, ·, and 1 keys; top left corner	0.1 mL
Site #2: Right half of mouse touch pad	0.1 mL
Site #3: Bottom left corner where palm rests	0.1 mL
Site #4: Bottom right corner where palm rests.	

☑ Using a 1-mL sterile syringe, inoculate each site with 0.1 mL of the working inoculum. Avoid stratification within the inoculum sample.

☑ Allow the inoculum to dry and record the drying times.

Device	Site	Dry time (min)	
Positive Device	Site #1	44.32	25.1°C /
	Site #2	42.24	
	Site #3	43.12	
	Site #4	45.18	
Test Device 1	Site #1	47.38	18.7°C / 20.2 °C
	Site #2	56.59	
	Site #3	55.02	
	Site #4	51.13	
Test Device 2	Site #1	46.43	25.3°C / 23.2°C
	Site #2	42.13	
	Site #3	49.13	
	Site #4	53.02	
Test Device 3	Site #1	47.13	22.3°C /
	Site #2	49.52	
	Site #3	44.25	
	Site #4	51.13	

Disinfection Procedure

☑ Positive Device: One contaminated device left unprocessed.

☑ Pushed the slide back into the unit until the front door is fully closed.

☑ Checked to make sure the keyboard was not blocking the light when placed back in the housing.

☑ Started timer to record disinfection time

Test Device	Disinfection time (seconds)		
Positive Device	N/A		
Test Device 1	90 sec	39.4	30 sec 1/2 = 45.1
Test Device 2	90 sec	42.2	1/2 = 46.4
Test Device 3	90 sec	37.2	1/2 = 45.7

Min 44.2 Max 46.1
44.0 46.4
44.0 45.7
↑ lost 5 sec
45.7 - 43.9

☐ After 90 seconds, turned off the power switch in the back of the unit.

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LOW LEVEL DISINFECTION EVALUATION VALIDATION FORMS

POSITIVE DEVICE

Date: 05/22/2019 – 05/23/2019

Organism Used: P. aeruginosa ATCC 9027

SPREAD PLATE DILUTION

Device	Vol. plated (mL)	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	CFU/mL
Positive Device Site #1	0.1 mL	/	>	>	155		/	1.55×10^6 1.48×10^6 1.52×10^6 $\bar{x} > 1.55\times10^6$
			>	>	148			
			>	>	152			
Positive Device Site #2	0.1 mL	/	>	210	92			$2.10\times10^{6.5}$ $2.0\times10^{6.5}$ $1.93\times10^{6.5}$ $\bar{x} = 2.01\times10^{6.5}$
	↓		>	206	101			
			>	193	87			
Positive Device Site #3	0.1 mL	/	>	121	31			1.21×10^5 1.38×10^5 1.27×10^5 $\bar{x} = 1.29\times10^5$
	↓		>	138	40			
			>	127	35			
Positive Device Site #4	0.1 mL	/	>	102	26			1.02×10^5 9.8×10^4 9.5×10^4 $\bar{x} = 9.8\times10^4$
	↓		>	98	31			
			>	95	22			

MEMBRANE FILTRATION

Device	Vol in 1g=1mL	1.0 mL	10 mL 1.0	CFU/mL
Positive Device Site #1	101.6	/	43	$=4.37\times10^4$ $=3.8\times10^4$ $=3.7\times10^4$
			38	
			37	
Positive Device Site #2	101.3	/	42	$=4.2\times10^4$ $=3.5\times10^4$ $=3.2\times10^4$
			35	
			32	
Positive Device Site #3	102.3	/	28	$=2.8\times10^4$ $=4.5\times10^4$ $=4.8\times10^4$
			45	
			48	
Positive Device Site #4	101.1	/	29	$=2.9\times10^4$ $=3.2\times10^4$ $=3.9\times10^4$
			32	
			39 59	

DIRECT PLATING

Device	Vol. plated (mL)	10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	CFU/mL
Positive Device Site #1	0.02	>	>	>	51	11	0	$=2.55\times10^6$
		>	>	>	48	7	0	$=2.40\times10^6$
		>	>	>	55	13	1	$=2.75\times10^6$
Positive Device Site #2	0.02	>	>	>	38	15	1	$=1.90\times10^6$
		>	>	>	43	8	0	$=2.15\times10^6$
		>	>	>	45	10	1	$=2.25\times10^6$
Positive Device Site #3	0.02	>	>	>	89	21	8	$=4.45\times10^6$
		>	>	>	93	38	2	$=4.65\times10^6$
		>	>	>	>	15	3	$=7.50\times10^6$
Positive Device Site #4	0.02	>	>	>	36	10	1	$=1.80\times10^6$
		>	>	>	59	8	0	$=2.95\times10^6$
		>	>	>	42	9	0	$=2.10\times10^6$

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LOW LEVEL DISINFECTION EVALUATION
VALIDATION FORMS

TEST DEVICE 1

Date: 05/22/2017 – 05/23/2017
Organism Used: P. aeruginosa ATCC 9027

Device	Vol. plated	SPREAD PLATE DILUTION 10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	CFU/mL
TD 1 Site #1	0.1 mL	0	0	0				= 0
	0.1 mL	0	0	0				
	0.1 mL	0	0	0				
TD 1 Site #2	0.1 mL	0	0	0				= 0
	0.1 mL	0	0	0				
	0.1 mL	0	0	0				
TD 1 Site #3	0.1 mL	0	0	0				= 0
	0.1 mL	0	0	0				
	0.1 mL	0	0	0				
TD 1 Site #4	0.1 mL	0	0	0				= 0
	0.1 mL	0	0	0				
	0.1 mL	0	0	0				

Device	Vol in 1mL = 1g	MEMBRANE FILTRATION 1.0 mL	10 mL	CFU/mL
TD 1 Site #1	104.0	0	0	= 0 CFU/mL
		0	0	
		0	0	
TD 1 Site #2	103.8	0	0	= 0 CFU/mL
		0	0	
		0	0	
TD 1 Site #3	100.2	0	0	= 0
		0	0	
		0	0	
TD 1 Site #4	104.7	0	0	= 0
		0	0	
		0	0	

Device	Vol. plated	DIRECT PLATING 10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	CFU/mL
TD1 Site #1	0.020 mL	0	0	0				= 0
		0	0	0				
		0	0	0				
TD 1 Site #2	0.020 mL	0	0	0				= 0
		0	0	0				
		0	0	0				
TD 1 Site #3	0.020 mL	0	0	0				= 0
		0	0	0				
		0	0	0				
TD 1 Site #4	0.020mL	0	0	0				= 0
		0	0	0				
		0	0	0				

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LOW LEVEL DISINFECTION EVALUATION
VALIDATION FORMS

TEST DEVICE 2

Date: 05/22/2019

Organism Used: P. aeruginosa ATCC 9027

Device	Vol. plated	SPREAD PLATE DILUTION						
		10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	CFU/mL
TD 2 Site #1	0.1 mL	0	0	0				=0
	0.1 mL	0	0	0				
	0.1 mL	0	0	0				
TD 2 Site #2	0.1 mL	0	0	0				=0
	0.1 mL	0	0	0				
	0.1 mL	0	0	0				
TD 2 Site #3	0.1 mL	0	0	0				=0
	0.1 mL	0	0	0				
	0.1 mL	0	0	0				
TD 2 Site #4	0.1 mL	0	0	0				=0
	0.1 mL	0	0	0				
	0.1 mL	0	0	0				

Device	Vol in 1mL=19.	MEMBRANE FILTRATION		
		1.0 mL	10 mL	CFU/mL
TD 2 Site #1	102.7	0		=0
		0		
		0		
TD 2 Site #2	101.0	0		=0
		0		
		0		
TD 2 Site #3	99.8	0		=0
		0		
		0		
TD 2 Site #4	101.4	0		=0
		0		
		0		

Device	Vol. plated (mL)	DIRECT PLATING						
		10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	CFU/mL
TD 2 Site #1	0.02	0	0	0				=0
		0	0	0				
		0	0	0				
TD 2 Site #2	0.02	0	0	0				=0
		0	0	0				
		0	0	0				
TD 2 Site #3	0.02	0	0	0				=0
		0	0	0				
		0	0	0				
TD 2 Site #4	0.02	0	0	0				=0
		0	0	0				
		0	0	0				

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LOW LEVEL DISINFECTION EVALUATION
VALIDATION FORMS

TEST DEVICE 3

Date: 05/22/2017

Organism Used: P. aeruginosa ATCC 9027

Device	Vol. plated	SPREAD PLATE DILUTION						
		10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	CFU/mL
TD3 Site #1	0.1 mL	0	0	0				=0
	↓	0	0	0				
		0	0	0				
TD3 Site #2	0.1mL	0	0	0				=0
	↓	0	0	0				
		0	0	0				
TD3 Site #3	0.1mL	0	0	0				=0
	↓	0	0	0				
		0	0	0				
TD3 Site #4	0.1mL	0	0	0				=0
	↓	0	0	0				
		0	0	0				

Device	Vol in	MEMBRANE FILTRATION		
		1.0 mL	10 mL	CFU/mL
TD3 Site #1	102.4	0		=0
		0		
		0		
TD3 Site #2	100.3	0		=0
		0		
		0		
TD3 Site #3	100.4	0		=0
		0		
		0		
TD3 Site #4	101.2	0		=0
		0		
		0		

Device	Vol. plated (mL)	DIRECT PLATING						
		10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	CFU/mL
TD3 Site #1	0.02	0	0	0				=0
		0	0	0				
		0	0	0				
TD3 Site #2	0.02	0	0	0				=0
		0	0	0				
		0	0	0				
TD3 Site #3	0.02	0	0	0				=0
		0	0	0				
		0	0	0				
TD3 Site #4	0.02	0	0	0				=0
		0	0	0				
		0	0	0				

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