FINAL REPORT

LOW LEVEL DISINFECTION EVALUATION – VIOGUARD KEYBOARD

Subject:

Microbial disinfection test, VIOGUARD, Inc. keyboard

Staphylococcus aureus

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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 *Staphylococcus aureus* by the UVKB-50 V2 self-sanitizing keyboard 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 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 (MSAG, Hardy Diagnostic Lot# 17062). 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 MSAG (Hardy Diagnostic Lot# 17062) 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 MSAG (Hardy Diagnostic Lot# 17062) 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 MSAG 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 plate.

- *Diluent control*: an aliquot of PEPT was processed as a sample for each test, placed on a MSAG 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.
- *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 MSAG 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.

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.

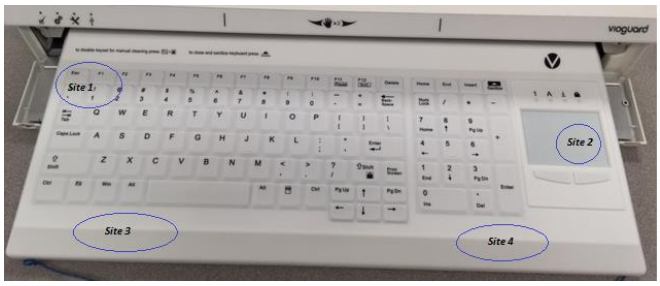
PROCEDURE

Test Device Inoculation

A 5% bovine serum solution was prepared using purified water (PURW) and inoculated with *Staphylococcus aureus* ATCC 25923 (*S. aureus*), 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 3.6 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	49.08
	2	42.15
	3	45.38
	4	47.13
Test 1	1	44.45
	2	42.15
	3	39.34
	4	43.50
Test 2	1	42.11
	2	40.35
	3	43.13
	4	45.82
Test 3	1	51.38
	2	42.15
	3	44.10
	4	45.58

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: 43.5 Max: 46.0
Test 2	Min: 43.2 Max: 46.1
Test 3	Min: 43.5 Max: 45.9

*Note: The last 30 seconds of the test, the power was between 43.5 -43.7 W

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. *Staphylococcus aureus* ATCC 25923 -SITE 1

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	<1.0 x 10^1	> 5.08	99.9992
2	<1.0 x 10^1	> 5.08	99.9992
3	<1.0 x 10^1	> 5.08	99.9992

Soil Titer: 3.6 x 10^7 CFU/mL
Positive Device Titer: 1.2 x 10^5 CFU/site 1

TABLE 3: DISINFECTION RESULTS. *Staphylococcus aureus* ATCC 25923 -SITE 2

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	<1.0 x 10^1	>5.10	99.9992
2	<1.0 x 10^1	>5.10	99.9992
3	<1.0 x 10^1	>5.10	99.9992

Soil Titer: 3.6 x 10^7 CFU/mL
Positive Device Titer: 1.25 x 10^5 CFU/site 2

TABLE 4: DISINFECTION RESULTS. *Staphylococcus aureus* ATCC 25923 -SITE 3

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	<1.0 x 10^1	> 5.09	99.9992
2	1.04 x 10^2	3.08	99.9161
3	<1.0 x 10^1	> 5.09	99.9992

Soil Titer: 3.6 x 10^7 CFU/mL
Positive Device Titer: 1.24 x 10^5 CFU/site 3

TABLE 5: DISINFECTION RESULTS. *Staphylococcus aureus* ATCC 25923 -SITE 4

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	<1.0 x 10^1	>5.10	99.9992
2	2.02 x 10^2	3.09	99.9181
3	<1.0 x 10^1	>5.10	99.9992

Soil Titer: 3.6 x 10^7 CFU/mL
Positive Device Titer: 1.27 x 10^5 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. *Staphylococcus aureus* ATCC 25923 -SITE 1

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	<1.0 x 10^1	>6.33	>99.9999
2	<1.0 x 10^1	>6.33	>99.9999
3	<1.0 x 10^1	>6.33	>99.9999

Soil Titer: 3.6 x 10^7 CFU/mL
Positive Device Titer: 2.14 x 10^6 CFU/site 1

TABLE 3: DISINFECTION RESULTS. *Staphylococcus aureus* ATCC 25923 -SITE 2

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	<1.0 x 10^1	>6.30	>99.9999
2	<1.0 x 10^1	>6.30	>99.9999
3	<1.0 x 10^1	>6.30	>99.9999

Soil Titer: 3.6 x 10^7 CFU/mL
Positive Device Titer: 2.0 x 10^6 CFU/site 2

TABLE 4: DISINFECTION RESULTS. *Staphylococcus aureus* ATCC 25923 -SITE 3

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	<1.0 x 10^1	>6.29	99.9999
2	<1.0 x 10^1	>6.29	99.9999
3	<1.0 x 10^1	>6.29	99.9999

Soil Titer: 3.6 x 10^7 CFU/mL
Positive Device Titer: 1.94 x 10^6 CFU/site 3

TABLE 5: DISINFECTION RESULTS. *Staphylococcus aureus* ATCC 25923 -SITE 4

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	<1.0 x 10^1	>6.30	>99.9999
2	<1.0 x 10^1	>6.30	>99.9999
3	<1.0 x 10^1	>6.30	>99.9999

Soil Titer: 3.6 x 10^7 CFU/mL
Positive Device Titer: 2.01 x 10^6 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: *Staphylococcus aureus* ATCC 25923

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Volume IN (mL)	Diluent Plated (mL)	CFU/plate	CFU/mL
1	100.3	0.1	109	1.09×10^5
2	100.3	0.1	133	1.33×10^5
3	100.3	0.1	118	1.18×10^5
		Positive Control Titer:		1.2×10^5
		Log 10		5.08

Site 1

Organism: *Staphylococcus aureus* ATCC 25923

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	100.1	<1	$<1.0 \times 10^1$	> 5.08	99.9992
2	100.1	<1	$<1.0 \times 10^1$	> 5.08	99.9992
3	101.1	<1	$<1.0 \times 10^1$	> 5.08	99.9992

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	74
CFU Plate 2:	71
CFU Plate 3:	73
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	3.6×10^7 CFU/mL

Positive control, **Site 2**

Organism: *Staphylococcus aureus* ATCC 25923

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Volume IN (mL)	Diluent Plated (mL)	CFU/plate	CFU/mL
1	100.9	0.1	121	1.21×10^5
2	100.9	0.1	138	1.38×10^5
3	100.9	0.1	117	1.17×10^5
		Positive Control Titer:		1.25×10^5
		Log 10		5.10

Site 2

Organism: *Staphylococcus aureus* ATCC 25923

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	100.3	<1	$<1.0 \times 10^1$	>5.10	99.9992
2	100.15	<1	$<1.0 \times 10^1$	>5.10	99.9992
3	100.14	<1	$<1.0 \times 10^1$	>5.10	99.9992

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	74
CFU Plate 2:	71
CFU Plate 3:	73
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	3.6×10^7 CFU/mL

Positive control, **Site 3**

Organism: *Staphylococcus aureus* ATCC 25923

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Volume IN (mL)	Diluent Plated (mL)	CFU/plate	CFU/mL
1	100.3	0.1	115	1.15×10^5
2	100.3	0.1	120	1.20×10^5
3	100.3	0.1	137	1.37×10^5
		Positive Control Titer:		1.24×10^5
		Log 10		5.09

Site 3

Organism: *Staphylococcus aureus* ATCC 25923

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	100.32	<1	$<1.0 \times 10^1$	> 5.09	99.9992
2	105.2	1	1.04×10^2	3.08	99.9161
3	109.3	<1	$<1.0 \times 10^1$	> 5.09	99.9992

CFU= Colony forming unit
< = None detected

um	
CFU Plate 1:	74
CFU Plate 2:	71
CFU Plate 3:	73
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	3.6×10^7 CFU/mL

Positive control, **Site 4**

Organism: *Staphylococcus aureus* ATCC 25923

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Volume IN (mL)	Diluent Plated (mL)	CFU/plate	CFU/mL
1	100.5	0.1	128	1.28×10^5
2	100.5	0.1	121	1.21×10^5
3	100.5	0.1	132	1.32×10^5
		Positive Control Titer:		1.27×10^5
		Log 10		5.10

Site 4

Organism: *Staphylococcus aureus* ATCC 25923

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	101.13	<1	$<1.0 \times 10^1$	>5.10	99.9992
2	100.8	2	2.02×10^2	3.09	99.9181
3	108.3	<1	$<1.0 \times 10^1$	>5.10	99.9992

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	74
CFU Plate 2:	71
CFU Plate 3:	73
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	3.6×10^7 CFU/mL

SPREAD PLATING DATA

Positive control, **Site 1**

Organism: *Staphylococcus aureus* ATCC 25923

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Volume IN (mL)	Dilution factor	CFU/plate	CFU/mL
1	0.1	1:1000	210	2.1×10^6
2	0.1	1:1000	213	2.13×10^6
3	0.1	1:1000	220	2.20×10^6
		Positive Control Titer:		2.14×10^6
		Log 10		6.33

Site 1

Organism: *Staphylococcus aureus* ATCC 25923

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	$<1.0 \times 10^1$	>6.33	>99.9999
2	0.1	10^0	<1	$<1.0 \times 10^1$	>6.33	>99.9999
3	0.1	10^0	<1	$<1.0 \times 10^1$	>6.33	>99.9999

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	74
CFU Plate 2:	71
CFU Plate 3:	73
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	3.6×10^7 CFU/mL

Positive control, **Site 2**

Organism: *Staphylococcus aureus* ATCC 25923

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL
1	0.1	1:1000	210	2.10×10^6
2	0.1	1:1000	193	1.93×10^6
3	0.1	1:1000	199	1.99×10^6
		Positive Control Titer:		2.0×10^6
		Log 10		6.30

Site 2

Organism: *Staphylococcus aureus* ATCC 25923

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	$<1.0 \times 10^1$	>6.30	>99.9999
2	0.1	10^0	<1	$<1.0 \times 10^1$	>6.30	>99.9999
3	0.1	10^0	<1	$<1.0 \times 10^1$	>6.30	>99.9999

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	74
CFU Plate 2:	71
CFU Plate 3:	73
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	3.6×10^7 CFU/mL

Positive control, **Site 3**

Organism: *Staphylococcus aureus* ATCC 25923

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL
1	0.1	1:1000	189	1.89×10^6
2	0.1	1:1000	193	1.93×10^6
3	0.1	1:1000	199	1.99×10^6
		Positive Control Titer:		1.94×10^6
		Log 10		6.29

Site 3

Organism: *Staphylococcus aureus* ATCC 25923

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	$<1.0 \times 10^1$	>6.29	99.9999
2	0.1	10^0	<1	$<1.0 \times 10^1$	>6.29	99.9999
3	0.1	10^0	<1	$<1.0 \times 10^1$	>6.29	99.9999

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	74
CFU Plate 2:	71
CFU Plate 3:	73
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	3.6×10^7 CFU/mL

Positive control, **Site 4**

Organism: *Staphylococcus aureus* ATCC 25923

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL
1	0.1	1:1000	210	2.1×10^6
2	0.1	1:1000	201	2.01×10^6
3	0.1	1:1000	193	1.93×10^6
		Positive Control Titer:		2.01×10^6
		Log 10		6.30

Site 4

Organism: *Staphylococcus aureus* ATCC 25923

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	$<1.0 \times 10^1$	>6.30	>99.9999
2	0.1	10^0	<1	$<1.0 \times 10^1$	>6.30	>99.9999
3	0.1	10^0	<1	$<1.0 \times 10^1$	>6.30	>99.9999

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	74
CFU Plate 2:	71
CFU Plate 3:	73
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	3.6×10^7 CFU/mL

DIRECT PLATING DATA

Positive control, **Site 1**

Organism: *Staphylococcus aureus* ATCC 25923

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	100	5.0×10^6
2	0.02	1:1000	110	5.5×10^6
3	0.02	1:1000	108	5.4×10^6
		Positive Control Titer:		5.3×10^6
		Log 10		6.72

Site 1

Organism: *Staphylococcus aureus* ATCC 25923

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	$<1.0 \times 10^1$	>6.72	> 99.9999
2	0.02	10^0	<1	$<1.0 \times 10^1$	>6.72	> 99.9999
3	0.02	10^0	<1	$<1.0 \times 10^1$	>6.72	> 99.9999

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	74
CFU Plate 2:	71
CFU Plate 3:	73
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	3.6×10^7 CFU/mL

Positive control, **Site 2**

Organism: *Staphylococcus aureus* ATCC 25923

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	84	4.2×10^6
2	0.02	1:1000	76	3.8×10^6
3	0.02	1:1000	80	4.0×10^6
		Positive Control Titer:		4.0×10^6
		Log 10		6.60

Site 2

Organism: *Staphylococcus aureus* ATCC 25923

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	$<1.0 \times 10^1$	>6.60	>99.9999
2	0.02	10^0	<1	$<1.0 \times 10^1$	>6.60	>99.9999
3	0.02	10^0	<1	$<1.0 \times 10^1$	>6.60	>99.9999

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	74
CFU Plate 2:	71
CFU Plate 3:	73
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	3.6×10^7 CFU/mL

Positive control, **Site 3**

Organism: *Staphylococcus aureus* ATCC 25923

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	134	6.7×10^6
2	0.02	1:1000	128	6.4×10^6
3	0.02	1:1000	153	7.65×10^6
		Positive Control Titer:		6.9×10^6
		Log 10		6.84

Site 3

Organism: *Staphylococcus aureus* ATCC 25923

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	$<1.0 \times 10^1$	>6.84	>99.9999
2	0.02	10^0	<1	$<1.0 \times 10^1$	>6.84	>99.9999
3	0.02	10^0	<1	$<1.0 \times 10^1$	>6.84	>99.9999

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	74
CFU Plate 2:	71
CFU Plate 3:	73
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	3.6×10^7 CFU/mL

Positive control, **Site 4**

Organism: *Staphylococcus aureus* ATCC 25923

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	84	4.2×10^6
2	0.02	1:1000	89	4.45×10^6
3	0.02	1:1000	93	4.65×10^6
		Positive Control Titer:		4.43×10^6
		Log 10		6.65

Site 4

Organism: *Staphylococcus aureus* ATCC 25923

Number of devices tested: 1

Number of replicate tests: 3

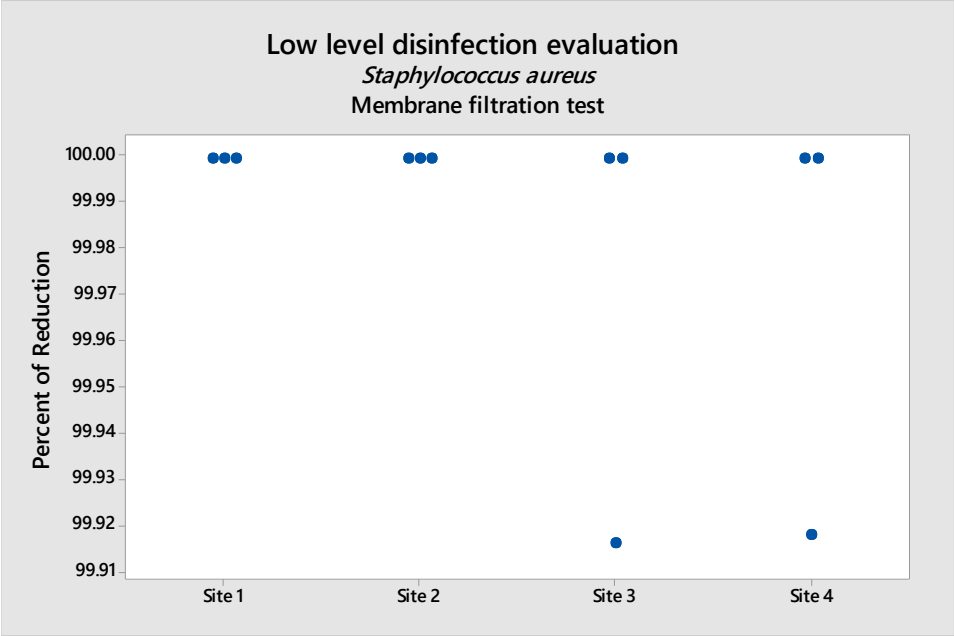
SITE 3 after 90-sec disinfection cycle

Device	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL	Log Reduction	Percent Reduction (%)
1	0.02	10^0	<1	$<1.0 \times 10^1$	>6.65	>99.9999
2	0.02	10^0	<1	$<1.0 \times 10^1$	>6.65	>99.9999
3	0.02	10^0	<1	$<1.0 \times 10^1$	>6.65	>99.9999

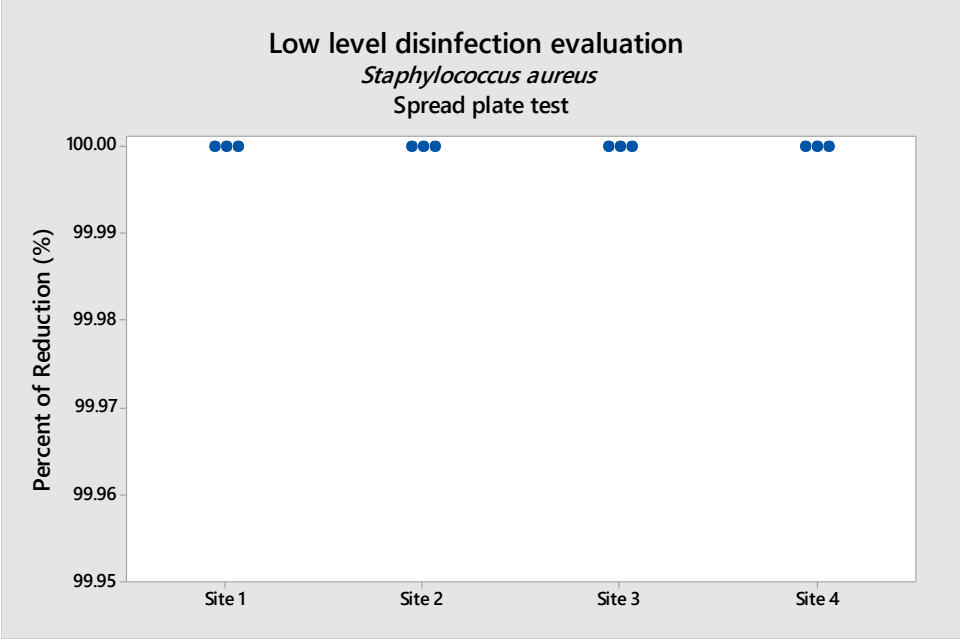
CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	74
CFU Plate 2:	71
CFU Plate 3:	73
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	3.6×10^7 CFU/mL

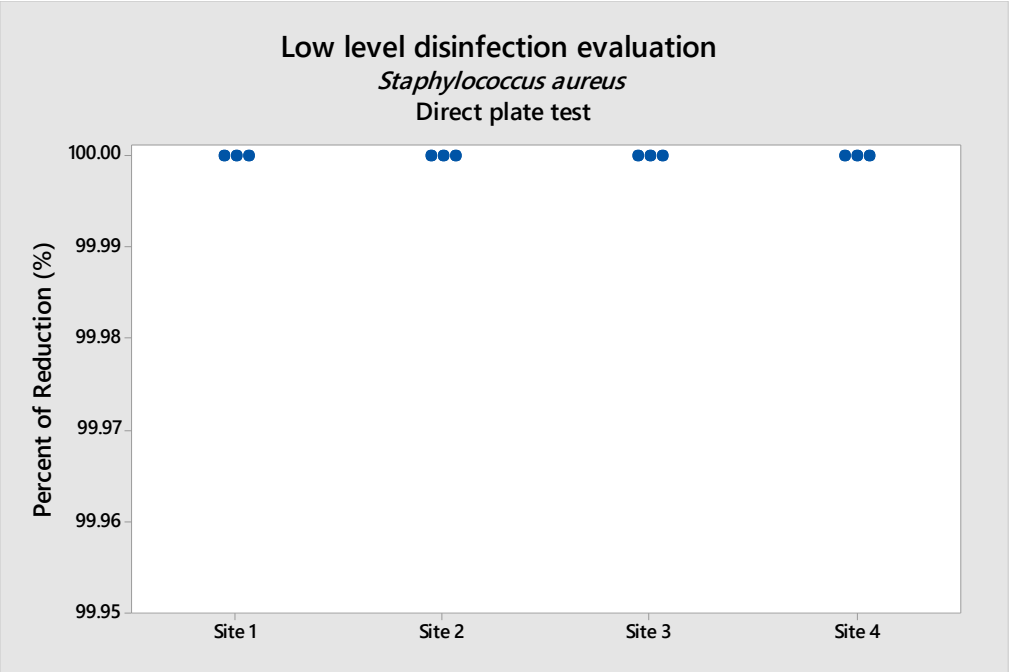
APPENDIX B: Graphics



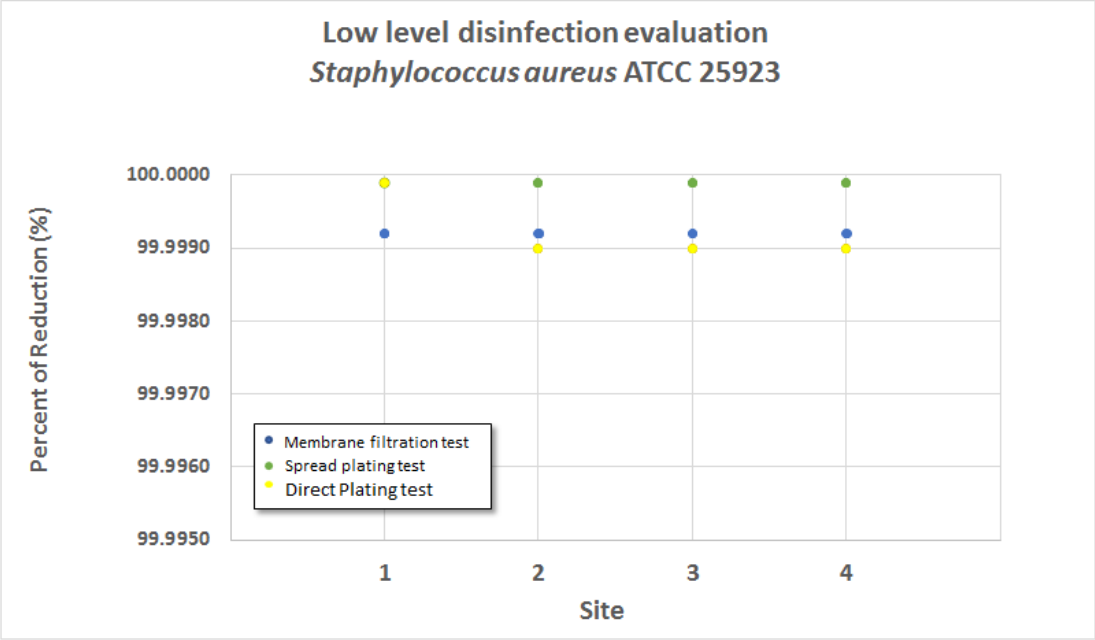
Graphic 1: Percent of reduction of low level disinfection evaluation of *Staphylococcus aureus* ATCC 25923. Membrane filtration test.



Graphic 2: Percent of reduction of low level disinfection evaluation of *Staphylococcus aureus* ATCC 25923. Spread Plating test.



Graphic 3: Percent of reduction of low level disinfection evaluation of *Staphylococcus aureus* ATCC 25923. Direct Plating



Graphic 4: Percent of reduction of low level disinfection evaluation of *Staphylococcus aureus* ATCC 25923

APPENDIX C: Raw Data

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LOW LEVEL DISINFECTION EVALUATION VALIDATION FORMS

Date: 23/May/2017 - 24/May/2017

Staphylococcus aureus ATCC 25923				
10^{-3}	10^{-4}	10^{-5}	CFU/mL	Average
>	74	18	(74+71+73)/3 x 10^4	
>	71	21	0.02	
>	73	20	=	36×10^7

CONTROL RESULTS:
MEDIA MSA
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 1 CFU/mL Site 4 0 CFU/mL
INCUBATOR: 0336 TEMP 36.6 °C

Date: _____

Pseudomonas aeruginosa ATCC 9077				
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: _____

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 _____

MEDIA

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

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LOW LEVEL DISINFECTION EVALUATION VALIDATION FORMS

Date: 05/22/2017 – 05/23/2017

Organism(s)	S. aureus
ATCC #	25923
Culture Age	18 hours
Plate Media/Lot#	TSA LOT #
Incubator ID#	0366
Temperature:	36.6°C

Time/Date In:	05/22/2017 4:55 pm
Time/Date Out:	05/23/2017 10:15 am

Inoculum preparation	
Abs at 420 nm	0.159
Prepared in/Lot #	S/ Bovine Serum
Spectrophotometer ID#	C0216 Thermo Scientific Genesys 10.

Equipment:

	Ondine ID#	Calibration verified	Additional equipment ☒ N/A
Micropipettes	C0034; 0368; C0030; C0012	☑ Yes ☐ N/A	
Vortex	C0075; C0073	☐ Yes ☑ N/A	
Timer	C0002	☑ 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	Criterion Jan-2020	MSAG Handy DX 2017-07.
Purified water	1610H29	Ricca OCT-2017	1mL syringe BD
Deionized water	VE 06040	Integ(a.	10mL syringe BD.
Bovine Serum	Cell supplemented w/iron	Hyclone Sandigm Jun/20	
Sterile swabs	4413	Puritan 10/20	
Filter	7053113	Pall	
Filter unit	N/A	Nalgene	
PBS	051M8212	Sigma-Aldrich.	
TSB	SLH7519	BD 2020-03	
Tween 80	0H270	Integ(a 2018-08	

Sample contamination

Number of devices contaminated: 1

Number of test: 4

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LOW LEVEL DISINFECTION EVALUATION VALIDATION FORMS

Sample contamination

S. aureus ATCC 25923.

☑ 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	0.1mL
Site #2: Right half of mouse touch pad	0.1mL
Site #3: Bottom left corner where palm rests	0.1mL
Site #4: Bottom right corner where palm rests.	0.1 mL.

☑ 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	49.08	23.8°C / 23.9°C.
	Site #2	42.15	
	Site #3	45.38	
	Site #4	47.13	
Test Device 1	Site #1	44.45	23.1°C / 23.8°C.
	Site #2	42.15	
	Site #3	39.34	
	Site #4	43.50	
Test Device 2	Site #1	42.11	24.1°C / 21.2
	Site #2	40.35	
	Site #3	43.13	
	Site #4	45.82	
Test Device 3	Site #1	51.38	24.4°C / 23.9
	Site #2	42.15	
	Site #3	44.10	
	Site #4	45.58	

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	41.7	30 sec = 46.0	Min = 43.5 Max = 46.0
Test Device 2	90 sec	41.2	30 sec = 46.1	Min = 43.52 Max = 46.1
Test Device 3	90 sec	41.8	30 sec = 45.7	Min = 43.5 Max = 45.9

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

Note: last 30 sec > 45.9 & 45.7

#43.2 Min TD02

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LOW LEVEL DISINFECTION EVALUATION VALIDATION FORMS

POSITIVE DEVICE

Date: 23-24 May 12017

Organism Used: S. aureus ATCC 25923.

Device	Vol. plated	SPREAD PLATE DILUTION 10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	CFU/mL
Positive Device Site #1	0.1mL	/	>	>	210	89	/	$=2.1\times10^6$
	↓	/	>	>	213	82	/	$=2.13\times10^6$
		/	>	>	220	93	/	$=2.2\times10^6$
								$\bar{x}=2.19\times10^6$
Positive Device Site #2	0.1mL	/	>	>	210	75	/	$=2.1\times10^6$
	↓	/	>	>	193	79	/	$=1.93\times10^6$
		/	>	>	199	85	/	$=1.99\times10^6$
								$\bar{x}=2.0\times10^6$
Positive Device Site #3	0.1mL	/	>	>	189	99	/	$=1.89\times10^6$
	↓	/	>	>	193	101	/	$=1.93\times10^6$
		/	>	>	199	83	/	$=1.99\times10^6$
								$\bar{x}=1.94\times10^6$
Positive Device Site #4	0.1mL	/	>	>	210	85	/	$=2.10\times10^6$
	↓	/	>	>	201	93	/	$=2.01\times10^6$
		/	>	>	193	95	/	$=1.93\times10^6$
								$\bar{x}=2.01\times10^6$

Device	Vol in	MEMBRANE FILTRATION 1.0 mL	10 mL 0.1	CFU/mL
Positive Device Site #1	100.3	/	109	$=1.09\times10^5$
		/	133	$=1.33\times10^5$
		/	118	$=1.18\times10^5$
				$\bar{x}=1.2\times10^5$
Positive Device Site #2	100.9	/	121	$=1.21\times10^5$
		/	138	$=1.38\times10^5$
		/	117	$=1.17\times10^5$
				$\bar{x}=1.25\times10^5$
Positive Device Site #3	100.3	/	115	$=1.15\times10^5$
		/	120	$=1.20\times10^5$
		/	137	$=1.37\times10^5$
				$\bar{x}=1.24\times10^5$
Positive Device Site #4	100.5	/	128	$=1.28\times10^5$
		/	121	$=1.21\times10^5$
		/	132	$=1.32\times10^5$
				$\bar{x}=1.27\times10^5$

Device	Vol. plated (mL)	DIRECT PLATING 10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	CFU/mL
Positive Device Site #1	0.02	/	/	/	100	22	1	5×10^6
		/	/	/	110	24	2	5.5×10^6
		/	/	/	108	20	1	5.4×10^6
								$\bar{x}=5.3\times10^6$
Positive Device Site #2	0.02	/	/	/	84	23	3	$=4.2\times10^6$
		/	/	/	76	21	2	$=3.8\times10^6$
		/	/	/	80	20	1	$=4\times10^6$
								$\bar{x}=4.0\times10^6$
Positive Device Site #3	0.02	/	/	/	134	18	2	$=6.7\times10^6$
		/	/	/	128	15	0	$=6.4\times10^6$
		/	/	/	153	17	1	$=7.65\times10^6$
								$\bar{x}=6.9\times10^6$
Positive Device Site #4	0.02	/	/	/	84	25	2	$=4.2\times10^6$
		/	/	/	89	21	1	$=4.45\times10^6$
		/	/	/	93	23	1	$=4.65\times10^6$
								$\bar{x}=4.43\times10^6$

24/May/16.

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LOW LEVEL DISINFECTION EVALUATION VALIDATION FORMS

TEST DEVICE 1

Date: 23/May/2017

Organism Used: S. aureus ATCC 25923

Device	Vol. plated	SPREAD PLATE DILUTION						
		10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	CFU/mL
TD 1 Site #1	0.1mL	0	0	0				=6
		0	0	0				
		0	0	0				
TD 1 Site #2	0.1mL	0	0	0				=0
		0	0	0				
		0	0	0				
TD 1 Site #3	0.1mL	0	0	0				=0
		0	0	0				
		0	0	0				
TD 1 Site #4	0.1mL	0	0	0				=0
		0	0	0				
		0	0	0				

Device	Vol in 1g = 1mL	MEMBRANE FILTRATION		
		1.0 mL	10 mL	CFU/mL
TD 1 Site #1	99.9	0	0	=0
		0	0	
		0	0	
TD 1 Site #2	100.5	0	0	=0
		0	0	
		0	0	
TD 1 Site #3	101.0	0	0	=0
		0	0	
		0	0	
TD 1 Site #4	100.1	0	0	=0
		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 1 Site #1	0.02	0	0	0				=0
		0	0	0				
		0	0	0				
TD 1 Site #2	0.02	0	0	0				=0
		0	0	0				
		0	0	0				
TD 1 Site #3	0.02	0	0	0				=0
		0	0	0				
		0	0	0				
TD 1 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 2

Date: 23-24 May 2017
Organism Used: S. aureus ATCC 25923

Device	Vol. plated	SPREAD PLATE DILUTION 10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	CFU/mL
TD2 Site #1	0.1mL ↓	0	0	0				=0
		0	0	0				
		0	0	0				
TD2 Site #2	0.1mL ↓	0	0	0				=0
		0	0	0				
		0	0	0				
TD2 Site #3	0.1mL ↓	0	0	0				=0
		0	0	0				
		0	0	0				
TD2 Site #4	0.1mL ↓	0	0	0				=0 =10 =10 >7 $\bar{x}$=<1
		1	0	0				
		1	0	0				

Device	Vol in	MEMBRANE FILTRATION 1.0 mL	10 mL	CFU/mL
TD2 Site #1	100.6	0		=0
		0		
		0		
TD2 Site #2	102.0	0		=0
		0		
		0		
TD2 Site #3	103.8	1		=104 =104 =104 >104
		1 $\bar{x}$=1		
		1		
TD2 Site #4	100.8	2		=202 =202 =202 >202
		2 $\bar{x}$=2		
		2		

Device	Vol. plated (mL)	DIRECT PLATING 10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	CFU/mL
TD2 Site #1	0.02	0	0	0				=0
		0	0	0				
		0	0	0				
TD2 Site #2	0.02	0	0	0				=0
		0	0	0				
		0	0	0				
TD2 Site #3	0.02	0	0	0				=0
		0	0	0				
		0	0	0				
TD2 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: 23-24 May/2017
Organism Used: S. aureus ATCC 25923

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

Device	Vol in 1g = 1mL	MEMBRANE FILTRATION 1.0 mL	10 mL	CFU/mL
TD Site #1	100.0	1	0	=100
		0	0	=0 $\bar{x}=33.$ <1
		0	0	=0
TD Site #2	100.1	0	0	=0
		1	0	=100 $\bar{x}=33$ <1
		0	0	=0
TD Site #3	100.3	0	0	=0
		0	0	=0
		0	0	=0
TD Site #4	103.7	0	0	=0
		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 Site #1	0.02	0	0	0				=0
		0	0	0				
		0	0	0				
TD Site #2	0.02	0	0	0				=0
		0	0	0				
		0	0	0				
TD Site #3	0.02	0	0	0				=0
		0	0	0				
		0	0	0				
TD Site #4	0.02	0	0	0				=0
		0	0	0				
		0	0	0				

END OF DOCUMENT