FINAL REPORT

LOW LEVEL DISINFECTION EVALUATION – VIOGUARD KEYBOARD

Subject:

Microbial disinfection test, VIOGUARD, Inc keyboard

Klebsiella pneumoniae

Document Date of Creation:

June 09, 2017

Date Studies Conducted:

June 01 – June 05, 2017

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

Tested by: *Cristina Romo*

Director: *David Meller*

Sponsor:

VIOGUARD, INC
19017 120TH AVE NE, STE 102
BOTHELL, WA 98011, USA

\

Contents

PURPOSE 3
METHODS 3
PROCEDURE 4
Test Device Inoculation 5
Disinfection Procedure 6
RESULTS 6
Membrane Filtration Test 6
TABLE 3: DISINFECTION RESULTS: *K. pneumoniae* ATCC 13883 6
Spread plate Test 7
TABLE 4: DISINFECTION RESULTS: *K. pneumoniae* ATCC 13883 7
CONCLUSIONS 8
APPENDIX A: Tabulated Raw Data 9
APPENDIX B: Graphics 22
APPENDIX C: Raw Data 23

PURPOSE

The purpose of this document is to report the test results of low-level disinfection of *Klebsiella pneumoniae* 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 MacConkey plate (Hardy Diagnostic Lot# 17096). 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 MacConkey plate (Hardy Diagnostic Lot# 17096) 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 MacConkey plate (Hardy Diagnostic Lot# 17096) 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 MacConkey 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.
- *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 MacConkey plate and incubated at 37°C, 5% CO_2 for 24 hours. No growth was observed on the negative media monitoring plate. o growth was observed.

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

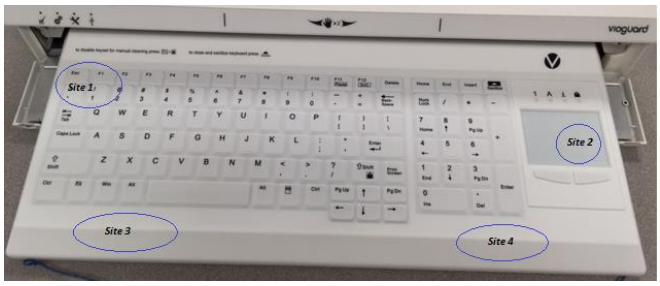
PROCEDURE

Test Device Inoculation

A 5% bovine serum solution was prepared using purified water (PURW) and inoculated with *Klebsiella pneumoniae* ATCC 13883 (*K. pneumoniae*), 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 1.5 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	49.12
	2	48.19
	3	42.13
	4	52.14
Test 1	1	53.12
	2	44.12
	3	45.17
	4	48.53
Test 2	1	49.12
	2	47.28
	3	42.38
	4	44.43
Test 3	1	53.12
	2	51.13
	3	55.38
	4	57.39

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: 40.3 Max: 42.0
Test 2	Min: 41.2 Max: 45.0
Test 3	Min: 41.0 Max: 45.3

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. *Klebsiella pneumoniae* ATCC 13883 -SITE 1

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	<1.0 x 10^0	>5.17	> 99.9993
2	<1.0 x 10^0	>5.17	> 99.9993
3	<1.0 x 10^0	>5.17	> 99.9993

Soil Titer: 1.50 x 10^7 CFU/mL
Positive Device Titer: 1.49 x 10^5 CFU/site 1

TABLE 3: DISINFECTION RESULTS. *Klebsiella pneumoniae* ATCC 13883 -SITE 2

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	<1.0 x 10^0	>5.15	> 99.9993
2	<1.0 x 10^0	>5.15	> 99.9993
3	<1.0 x 10^0	>5.15	> 99.9993

Soil Titer: 1.50 x 10^7CFU/mL
Positive Device Titer: 1.42 x 10^5 CFU/site 2

TABLE 4: DISINFECTION RESULTS. *Klebsiella pneumoniae* ATCC 13883 -SITE 3

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	<1.0 x 10^0	>5.15	> 99.9993
2	<1.0 x 10^0	>5.15	> 99.9993
3	<1.0 x 10^0	>5.15	> 99.9993

Soil Titer 1.50 x 10^7 CFU/mL
Positive Device Titer: 1.42 x 10^5 CFU/site 3

TABLE 5: DISINFECTION RESULTS. *Klebsiella pneumoniae* ATCC 13883 -SITE 4

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	<1.0 x 10^0	>5.14	> 99.9993
2	<1.0 x 10^0	>5.14	> 99.9993
3	<1.0 x 10^0	>5.14	> 99.9993

Soil Titer: 1.50 x 10^7 CFU/mL
Positive Device Titer: 1.38 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. *Klebsiella pneumoniae* ATCC 13883 -SITE 1

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	$<1.0 \times 10^0$	>5.12	>99.9992
2	$<1.0 \times 10^0$	>5.12	>99.9992
3	$<1.0 \times 10^0$	>5.12	>99.9992

Soil Titer: 1.5×10^7 CFU/mL
Positive Device Titer: 1.33×10^5 CFU/site 1

TABLE 3: DISINFECTION RESULTS. *Klebsiella pneumoniae* ATCC 13883 -SITE 2

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	$<1.0 \times 10^0$	>5.13	>99.9993
2	$<1.0 \times 10^0$	>5.13	>99.9993
3	$<1.0 \times 10^0$	>5.13	>99.9993

Soil Titer: 1.5×10^7 CFU/mL
Positive Device Titer: 1.35×10^5 CFU/site 2

TABLE 4: DISINFECTION RESULTS. *Klebsiella pneumoniae* ATCC 13883 -SITE 3

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	$<1.0 \times 10^0$	>5.08	>99.9992
2	$<1.0 \times 10^0$	>5.09	>99.9992
3	$<1.0 \times 10^0$	>5.09	>99.9992

Soil Titer: 1.5×10^7 CFU/mL
Positive Device Titer: 1.22×10^5 CFU/site 3

TABLE 5: DISINFECTION RESULTS. *Klebsiella pneumoniae* ATCC 13883-SITE 4

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	$<1.0 \times 10^0$	>5.16	>99.9993
2	$<1.0 \times 10^0$	>5.16	>99.9993
3	$<1.0 \times 10^0$	>5.16	>99.9993

Soil Titer: 1.5×10^7 CFU/mL
Positive Device Titer: 1.46×10^5 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: *Klebsiella pneumoniae* ATCC 13883

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Volume IN (mL)	Diluent Plated (mL)	CFU/plate	CFU/mL
1	100.2	0.1	139	1.39×10^5
2	100.2	0.1	148	1.48×10^5
3	100.2	0.1	159	1.59×10^5
		Positive Control Titer:		1.49×10^5
		Log 10		5.17

Site 1

Organism: *Klebsiella pneumoniae* ATCC 13883

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	100.2	<1	$<1.0 \times 10^0$	>5.17	> 99.9993
2	100.2	<1	$<1.0 \times 10^0$	>5.17	> 99.9993
3	102.9	<1	$<1.0 \times 10^0$	>5.17	> 99.9993

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	30
CFU Plate 2:	28
CFU Plate 3:	34
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	1.50×10^7 CFU/mL

Positive control, **Site 2**

Organism: *Klebsiella pneumoniae* ATCC 13883

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Volume IN (mL)	Diluent Plated (mL)	CFU/plate	CFU/mL
1	100.1	0.1	138	1.38×10^5
2	100.1	0.1	142	1.42×10^5
3	100.1	0.1	145	1.45×10^5
		Positive Control Titer:		1.42×10^5
		Log 10		5.15

Site 2

Organism: *Klebsiella pneumoniae* ATCC 13883

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	100.8	<1	$<1.0 \times 10^0$	>5.15	> 99.9993
2	100.8	<1	$<1.0 \times 10^0$	>5.15	> 99.9993
3	101.5	<1	$<1.0 \times 10^0$	>5.15	> 99.9993

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	30
CFU Plate 2:	28
CFU Plate 3:	34
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	1.50×10^7 CFU/mL

Positive control, **Site 3**

Organism: *Klebsiella pneumoniae* ATCC 13883

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Volume IN (mL)	Diluent Plated (mL)	CFU/plate	CFU/mL
1	100.8	0.1	138	1.38×10^5
2	100.8	0.1	142	1.42×10^5
3	100.8	0.1	145	1.45×10^5
		Positive Control Titer:		1.42×10^5
		Log 10		5.15

Site 3

Organism: *Klebsiella pneumoniae* ATCC 13883

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	101.3	<1	$<1.0 \times 10^0$	>5.15	> 99.9993
2	102.3	<1	$<1.0 \times 10^0$	>5.15	> 99.9993
3	101.3	<1	$<1.0 \times 10^0$	>5.15	> 99.9993

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	30
CFU Plate 2:	28
CFU Plate 3:	34
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	1.50×10^7 CFU/mL

Positive control, **Site 4**

Organism: *Klebsiella pneumoniae* ATCC 13883

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Volume IN (mL)	Diluent Plated (mL)	CFU/plate	CFU/mL
1	103.1	0.1	144	1.44×10^5
2	103.1	0.1	137	1.37×10^5
3	103.1	0.1	133	1.33×10^5
		Positive Control Titer:		1.38×10^5
		Log 10		5.14

Site 4

Organism: *Klebsiella pneumoniae* ATCC 13883

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	102.3	<1	$<1.0 \times 10^0$	>5.14	>99.9993
2	101.8	<1	$<1.0 \times 10^0$	>5.14	>99.9993
3	100.7	<1	$<1.0 \times 10^0$	>5.14	>99.9993

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	30
CFU Plate 2:	28
CFU Plate 3:	34
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	1.50×10^7 CFU/mL

SPREAD PLATING DATA

Positive control, **Site 1**

Organism: *Klebsiella pneumoniae* ATCC 13883

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Volume IN (mL)	Dilution factor	CFU/plate	CFU/mL
1	0.1	1:100	129	1.29×10^5
2	0.1	1:100	132	1.32×10^5
3	0.1	1:100	137	1.37×10^5
		Positive Control Titer:		1.33×10^5
		Log 10		5.12

Site 1

Organism: *Klebsiella pneumoniae* ATCC 13883

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	$<1.0 \times 10^0$	>5.12	>99.9992
2	0.1	10^0	<1	$<1.0 \times 10^0$	>5.12	>99.9992
3	0.1	10^0	<1	$<1.0 \times 10^0$	>5.12	>99.9992

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	30
CFU Plate 2:	28
CFU Plate 3:	34
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	1.50×10^7 CFU/mL

Positive control, **Site 2**

Organism: *Klebsiella pneumoniae* ATCC 13883

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	128	1.28×10^5
2	0.1	1:100	139	1.39×10^5
3	0.1	1:100	138	1.38×10^5
		Positive Control Titer:		1.35×10^5
		Log 10		5.13

Site 2

Organism: *Klebsiella pneumoniae* ATCC 13883

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	$<1.0 \times 10^0$	>5.13	>99.9993
2	0.1	10^0	<1	$<1.0 \times 10^0$	>5.13	>99.9993
3	0.1	10^0	<1	$<1.0 \times 10^0$	>5.13	>99.9993

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	30
CFU Plate 2:	28
CFU Plate 3:	34
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	1.50×10^7 CFU/mL

Positive control, **Site 3**

Organism: *Klebsiella pneumoniae* ATCC 13883

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	129	1.29×10^5
2	0.1	1:100	117	1.17×10^5
3	0.1	1:100	121	1.21×10^5
		Positive Control Titer:		1.22×10^5
		Log 10		5.09

Site 3

Organism: *Klebsiella pneumoniae* ATCC 13883

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	$<1.0 \times 10^0$	>5.08	>99.9992
2	0.1	10^0	<1	$<1.0 \times 10^0$	>5.09	>99.9992
3	0.1	10^0	<1	$<1.0 \times 10^0$	>5.09	>99.9992

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	30
CFU Plate 2:	28
CFU Plate 3:	34
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	1.50×10^7 CFU/mL

Positive control, **Site 4**

Organism: *Klebsiella pneumoniae* ATCC 13883

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	151	1.51×10^5
2	0.1	1:100	142	1.42×10^5
3	0.1	1:100	144	1.44×10^5
		Positive Control Titer:		1.46×10^5
		Log 10		5.16

Site 4

Organism: *Klebsiella pneumoniae* ATCC 13883

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	$<1.0 \times 10^0$	>5.16	>99.9993
2	0.1	10^0	<1	$<1.0 \times 10^0$	>5.16	>99.9993
3	0.1	10^0	<1	$<1.0 \times 10^0$	>5.16	>99.9993

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	30
CFU Plate 2:	28
CFU Plate 3:	34
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	1.50×10^7 CFU/mL

DIRECT PLATING DATA

Positive control, **Site 1**

Organism: *Klebsiella pneumoniae* ATCC 13883

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	29	1.45×10^6
2	0.02	1:1000	33	1.65×10^6
3	0.02	1:1000	35	1.75×10^6
		Positive Control Titer:		1.62×10^6
		Log 10		6.21

Site 1

Organism: *Klebsiella pneumoniae* ATCC 13883

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	$<1.0 \times 10^0$	>6.21	>99.9999
2	0.02	10^0	<1	$<1.0 \times 10^0$	>6.21	>99.9999
3	0.02	10^0	<1	$<1.0 \times 10^0$	>6.21	>99.9999

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	30
CFU Plate 2:	28
CFU Plate 3:	34
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	1.50×10^7 CFU/mL

Positive control, **Site 2**

Organism: *Klebsiella pneumoniae* ATCC 13883

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	28	1.4×10^6
2	0.02	1:1000	34	1.7×10^6
3	0.02	1:1000	33	1.65×10^6
		Positive Control Titer:		1.58×10^6
		Log 10		6.20

Site 2

Organism: *Klebsiella pneumoniae* ATCC 13883 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	$<1.0 \times 10^0$	>6.20	>99.9999
2	0.02	10^0	<1	$<1.0 \times 10^0$	>6.20	>99.9999
3	0.02	10^0	<1	$<1.0 \times 10^0$	>6.20	>99.9999

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	30
CFU Plate 2:	28
CFU Plate 3:	34
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	1.50×10^7 CFU/mL

Positive control, **Site 3**

Organism: *Klebsiella pneumoniae* ATCC 13883

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	29	1.45×10^6
2	0.02	1:1000	22	1.10×10^6
3	0.02	1:1000	21	1.05×10^6
		Positive Control Titer:		1.20×10^6
		Log 10		6.08

Site 3

Organism: *Klebsiella pneumoniae* ATCC 13883

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	$<1.0 \times 10^0$	>6.08	>99.9999
2	0.02	10^0	<1	$<1.0 \times 10^0$	>6.08	>99.9999
3	0.02	10^0	<1	$<1.0 \times 10^0$	>6.08	>99.9999

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	30
CFU Plate 2:	28
CFU Plate 3:	34
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	1.50×10^7 CFU/mL

Positive control, **Site 4**

Organism: *Klebsiella pneumoniae* ATCC 13883

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	32	1.60×10^5
2	0.02	1:1000	21	1.05×10^6
3	0.02	1:1000	25	1.25×10^6
		Positive Control Titer:		1.30×10^6
		Log 10		6.11

Site 4

Organism: *Klebsiella pneumoniae* ATCC 13883

Number of devices tested: 1

Number of replicate tests: 3

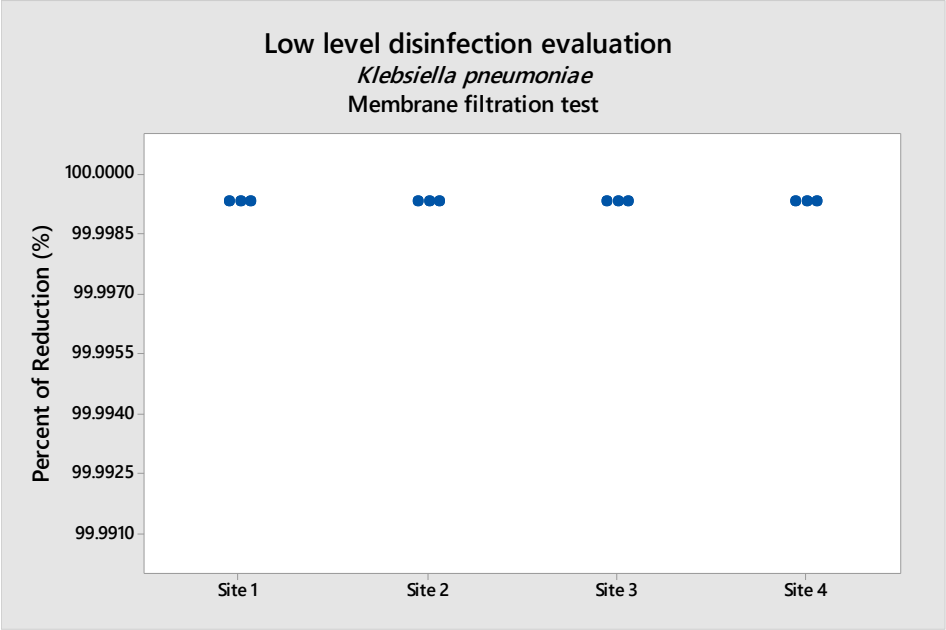
SITE 3 after 90-sec disinfection cycle

Device	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL	Log Reduction	Percent Reduction (%)
1	0.02	10^0	<1	$<1.0 \times 10^0$	>6.11	>99.9999
2	0.02	10^0	<1	$<1.0 \times 10^0$	>6.11	>99.9999
3	0.02	10^0	<1	$<1.0 \times 10^0$	>6.11	>99.9999

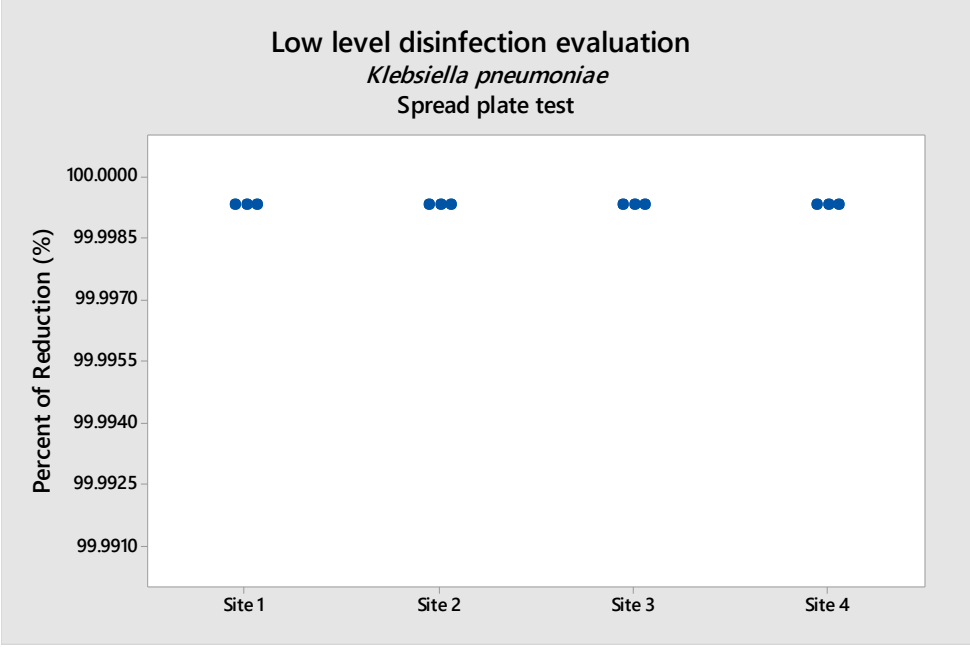
CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	30
CFU Plate 2:	28
CFU Plate 3:	34
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	1.50×10^7 CFU/mL

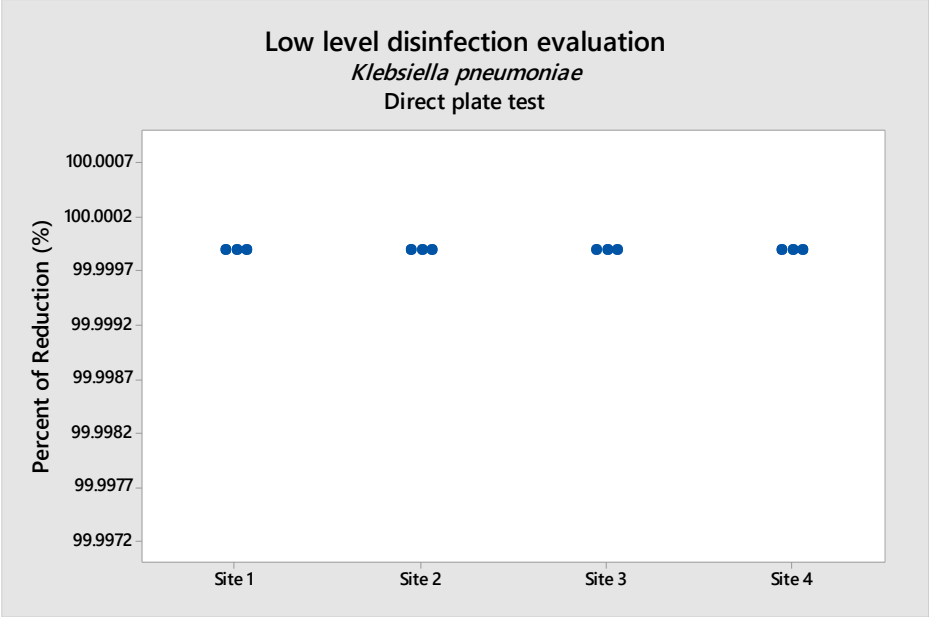
APPENDIX B: Graphics



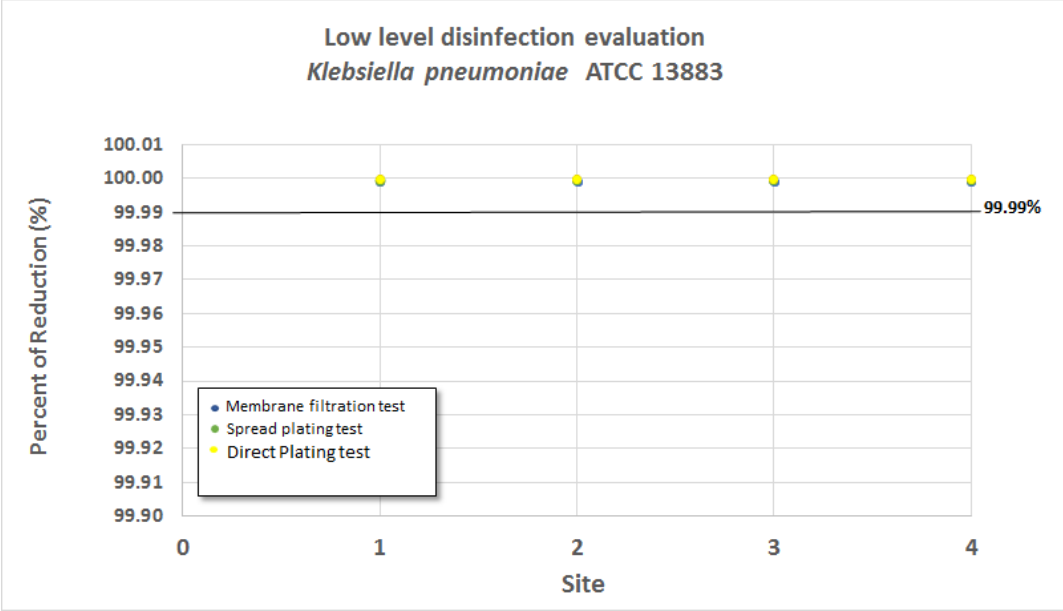
Graphic 1: Percent of reduction of low level disinfection evaluation of *Klebsiella pneumoniae* ATCC 13883. Membrane filtration test.



Graphic 2: Percent of reduction of low level disinfection evaluation of *Klebsiella pneumoniae* ATCC 13883. Spread Plating test.



Graphic 3: Percent of reduction of low level disinfection evaluation of *Klebsiella pneumoniae* ATCC 13883. Direct Plating



Graphic 4: Percent of reduction of low level disinfection evaluation of *Klebsiella pneumoniae* ATCC 13883

ondine

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: _____

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: 06/05/2017

Klebsiella pneumoniae ATCC 13883

10^{-3}	10^{-4}	10^{-5}	CFU/mL	Average
>	30	2		1.5×10^7
>	28	1		
>	34	3		

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:

☑ MEMBRANE FILTRATION
☑ DIRECT PLATING
☑ SPREAD PLATE: AMOUNT PLATED_____

MEDIA

☐ TSA
☑ TSB
☐ MSA
☐ EMB
☑ MAC CONKEY
☐ CETRAMIDE
☐ MUELLER HINTON
☐ OTHER

ondine

LOW LEVEL DISINFECTION EVALUATION VALIDATION FORMS

Date: 06/01/17

Organism(s)	K. pneumoniae
ATCC #	13883
Culture Age	18 hours
Plate Media/Lot#	TSA LOT # 051617
Incubator ID#	0336
Temperature:	36.6°C

Time/Date In:	06/01/17	8:30 pm
Time/Date Out:	06/02/17	9:49 am

Inoculum preparation	
Abs at 420 nm	0.118
Prepared in/Lot #	5% Bovine Serum
Spectrophotometer ID#	C0216 Genesys 10 S

Equipment:

	Ondine ID#	Calibration verified	Additional equipment ☐ N/A
Micropipettes	C0034; 0388; C0030	☑ Yes ☐ N/A	
Vortex	C0015, C0073	☐ Yes ☑ N/A	
Timer	C0202	☑ Yes ☐ N/A	
Biosafety Cabinet/Hood	0388	☑ Yes ☐ N/A	
Balance	0350, C0066	☑ Yes ☐ N/A	
Thermometer	C0203	☑ Yes ☐ N/A	
Aspirator pipet	C0205 C0092	☐ Yes ☑ N/A	
Colony counter	S/N	☐ Yes ☑ N/A	

Supplies	LOT #	EXP /Manufacturer	Additional equipment ☐ N/A
Peptone-Tween water	17075	Criterion Jan 20	McConkey Criterion
Purified water	1610889	Rec'd 10-17	1 mL BD syringe
Deionized water	VE06040	Integra	10 mL BD syringe
Bovine Serum	14A15	Invitrogen Jan 20	
Sterile swabs	4413	Puritan 10-20	
Filter	7053113	Pall	
Filter unit	N/A	Nalgene	
PBS	051M8212	Sigma	
TSB	51A519	BD - 03-20	
Tween 80	CH274118	Integra 08-2018	

Sample contamination

Number of devices contaminated: 1

Number of test: 4

ondine

LOW LEVEL DISINFECTION EVALUATION
VALIDATION FORMS

Sample contamination

K-pneumoniae ATCC 13883

☑ 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.	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	48:12
	Site #2	48:19
	Site #3	42:13
	Site #4	52:14
Test Device 1	Site #1	53:12
	Site #2	44:12
	Site #3	45:13
	Site #4	48:53
Test Device 2	Site #1	48:12
	Site #2	43:28
	Site #3	42:38
	Site #4	44:45
Test Device 3	Site #1	53:12
	Site #2	51:13
	Site #3	50:38
	Site #4	54:53

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
Test Device 2	90 sec
Test Device 3	90 sec

Timer used

40.3 - 44.0
41.2 - 45.0
41.0 - 45.3

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

ondine

LOW LEVEL DISINFECTION EVALUATION
VALIDATION FORMS

POSITIVE DEVICE

Date: 02-05 June / 2017

Organism Used: E. gallinarum ATCC 13825

Device	Vol. plated	SPREAD PLATE DILUTION						CFU/mL
		10^{2}	10^{3}	10^{4}	10^{5}	10^{6}	10^{7}	
Positive Device Site #1	0.1mL			129	10	0		1.29×10^{5}
	↓			132	8	0		1.32×10^{5}
				137	1	0		1.37×10^{5}
Positive Device Site #2	0.1mL			134	3	0		1.34×10^{5}
	↓			136	2	0		1.36×10^{5}
				138	2	0		1.38×10^{5}
Positive Device Site #3	0.1mL			129	1	0		1.29×10^{5}
	↓			117	3	0		1.17×10^{5}
				121	1	0		1.21×10^{5}
Positive Device Site #4	0.1mL			151	8	0		1.51×10^{5}
	↓			142	9	0		1.42×10^{5}
				144	13	0		1.44×10^{5}

Device	Vol in	MEMBRANE FILTRATION		
		1.0 mL: 0.1	10 mL	CFU/mL
Positive Device Site #1	$10^{0}.2$	139		1.39×10^{3}
		148		1.48×10^{3}
		155		1.55×10^{3}
Positive Device Site #2	$10^{0}.1$	138		1.38×10^{3}
		142		1.42×10^{3}
		147		1.47×10^{3}
Positive Device Site #3	$10^{0}.5$	138		1.38×10^{3}
		142		1.42×10^{3}
		145		1.45×10^{3}
Positive Device Site #4	$10^{1}.2$	144		1.44×10^{4}
		139		1.39×10^{4}
		133		1.33×10^{4}

Device	Vol. plated	DIRECT PLATING						CFU/mL
		10^{1}	10^{2}	10^{3}	10^{4}	10^{5}	10^{6}	
Positive Device Site #1	0.02				29	2	0	1.45×10^{6}
					33	1	0	1.63×10^{6}
					35	0	0	1.75×10^{6}
Positive Device Site #2	0.02				25	2	0	1.4×10^{6}
					34	2	0	1.7×10^{6}
					33	1	0	1.65×10^{6}
Positive Device Site #3	0.02				29	1	0	1.45×10^{6}
					22	0	0	1.1×10^{6}
					21	1	0	1.05×10^{6}
Positive Device Site #4	0.02				32	3	0	1.6×10^{6}
					21	5	0	1.05×10^{6}
					25	1	0	1.25×10^{6}

ondine

LOW LEVEL DISINFECTION EVALUATION
VALIDATION FORMS

TEST DEVICE 1

Date: 02 June - 03 June

Organism Used: E. coli ATCC #8739

Device	Vol. plated	SPREAD PLATE DILUTION						
		10^0	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	CFU/mL
TD1 Site #1	0.1 mL	0	→	→				0
	↓	0	→	→				
		0	→	→				
TD1 Site #2	0.1 mL	0	→	→				0
	↓	0	→	→				
		0	→	→				
TD1 Site #3	0.1 mL	0	→	→				0
	↓	0	→	→				
		0	→	→				
TD1 Site #4	0.1 mL	0	→	→				0
	↓	0	→	→				
		0	→	→				

Device	Vol in	MEMBRANE FILTRATION		
		1.0 mL	10 mL	CFU/mL
TD1 Site #1	100.2	0	0	0
		0	0	
		0	0	
TD1 Site #2	100.4	0	0	0
		0	0	
		0	0	
TD1 Site #3	100.3	0	0	0
		0	0	
		0	0	
TD1 Site #4	100.3	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
TD1 Site #1	0.02	0	→	→				0
		0	→	→				
		0	→	→				
TD1 Site #2	0.02	0	→	→				0
		0	→	→				
		0	→	→				
TD1 Site #3	0.02	0	→	→				0
		0	→	→				
		0	→	→				
TD1 Site #4	0.02	0	→	→				0
		0	→	→				
		0	→	→				

ondine

LOW LEVEL DISINFECTION EVALUATION
VALIDATION FORMS

TEST DEVICE 2

Date: 02 June - 05 June

Organism Used: K. pneumoniae 3513

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
TD 2 Site #2	0.1 mL	0	0	0				0
TD 2 Site #3	0.1 mL	0	0	0				0
TD 2 Site #4	0.1 mL	0	0	0				0

Device	Vol in	MEMBRANE FILTRATION 1.0 mL	10 mL	CFU/mL
TD 2 Site #1	100.2	0	0	0
TD 2 Site #2	100.3	0	0	0
TD 2 Site #3	102.3	0	0	0
TD 2 Site #4	101.3	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
TD 2 Site #2	0.02	0						0
TD 2 Site #3	0.02	0						0
TD 2 Site #4	0.02	0						0

ondine

LOW LEVEL DISINFECTION EVALUATION
VALIDATION FORMS

TEST DEVICE 3

Date: 02 June / 05 June 17
Organism Used: K. pneumoniae ATCC 13883

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

Device	Vol in 10^{-5} tube	MEMBRANE FILTRATION 1.0 mL	10 mL	CFU/mL
TD Site #1	102.9	0	0	0
	↓	↓	↓	
TD Site #2	101.5	0	0	0
	↓	↓	↓	
TD Site #3	101.3	0	0	0
	↓	↓	↓	
TD Site #4	100.7	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	→	→				
TD Site #2	0.02	0	→	→				0
		0	→	→				
		0	→	→				
TD Site #3	0.02	0	→	→				0
		0	→	→				
		0	→	→				
TD Site #4	0.02	0	→	→				0
		0	→	→				
		0	→	→				

END OF DOCUMENT