FINAL REPORT

LOW LEVEL DISINFECTION EVALUATION – VIOGUARD KEYBOARD

Subject:

Microbial disinfection test, VIOGUARD, Inc keyboard

Escherichia coli

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

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Sponsor:

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Contents

PURPOSE 3

METHODS 3

PROCEDURE 5

Test Device Inoculation 5

Disinfection Procedure 6

RESULTS 7

Membrane Filtration test: 7

TABLE 3: DISINFECTION RESULTS. *Escherichia coli* ATCC 25922 7

Spread plate test: 8

TABLE 4: DISINFECTION RESULTS. *Escherichia coli* ATCC 25922 8

CONCLUSIONS 9

APPENDIX A: Tabulated Raw Data 10

APPENDIX B: Graphics 22

APPENDIX C: Raw Data 24

PURPOSE

The purpose of this document is to report the test results of low-level disinfection of *Escherichia coli* 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 an eosin methylene blue agar (EMB, Hardy Diagnostic Lot# 0871704). 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 eosin methylene blue agar (EMB, Hardy Diagnostic Lot#0871704) 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 eosin methylene blue agar (EMB, Hardy Diagnostic Lot#0871704) 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*: An EMB 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 an EMB plate and incubated at 37°C, 5% CO_2 for 24 hours. No growth was observed.
- *Diluent control*: an aliquot of PEPT was processed as a sample for each test, placed on an EMB 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 an EMB 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.

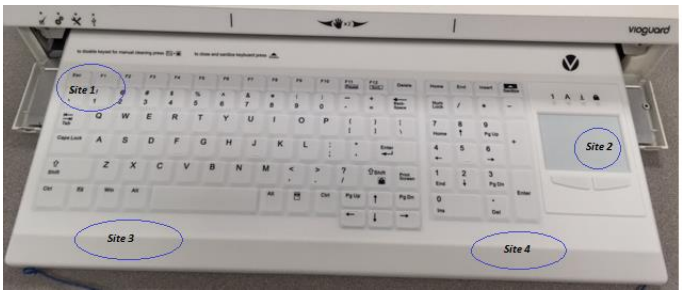
PROCEDURE

Test Device Inoculation

A 5% bovine serum solution was prepared using purified water (PURW) and inoculated with *Escherichia coli* ATCC 25922 (*E. coli*), 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 2.2 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	48.20
	2	45.13
	3	51.10
	4	47.13
Test 1	1	52.38
	2	59.15
	3	47.12
	4	49.58
Test 2	1	43.18
	2	49.15
	3	42.20
	4	45.23
Test 3	1	51.13
	2	45.15
	3	39.18
	4	43.21

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: 41.3 Max: 46.3
Test 2	Min: 40.1 Max: 46.0
Test 3	Min: 39.1 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. *Escherichia coli* ATCC 25922 -SITE 1

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

Soil Titer: 2.2×10^7 CFU/mL
Positive Device Titer: 1.47×10^5 CFU/site 1

TABLE 3: DISINFECTION RESULTS. *Escherichia coli* ATCC 25922 -SITE 2

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	$<1.0 \times 10^1$	> 5.28	99.9995
2	$<1.0 \times 10^1$	> 5.28	99.9995
3	$<1.0 \times 10^1$	> 5.28	99.9995

Soil Titer: 2.2×10^7 CFU/mL
Positive Device Titer: 1.92×10^5 CFU/site 2

TABLE 4: DISINFECTION RESULTS. *Escherichia coli* ATCC 25922 -SITE 3

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	$<1.0 \times 10^1$	> 5.31	99.9995
2	$<1.0 \times 10^1$	> 5.31	99.9995
3	$<1.0 \times 10^1$	> 5.31	99.9995

Soil Titer: 2.2×10^7 CFU/mL
Positive Device Titer: 2.05×10^5 CFU/site 3

TABLE 5: DISINFECTION RESULTS. *Escherichia coli* ATCC 25922 -SITE 4

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	$<1.0 \times 10^1$	> 5.28	99.9995
2	$<1.0 \times 10^1$	> 5.28	99.9995
3	$<1.0 \times 10^1$	> 5.28	99.9995

Soil Titer: 2.2×10^7 CFU/mL
Positive Device Titer: 1.92×10^5 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. *Escherichia coli* ATCC 25922 -SITE 1

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	<1.0 x 10^1	> 5.21	99.9994
2	<1.0 x 10^1	> 5.21	99.9994
3	<1.0 x 10^1	> 5.21	99.9994

Soil Titer: 2.2 x 10^7 CFU/mL
Positive Device Titer: 1.62 x 10^5 CFU/site 1

TABLE 3: DISINFECTION RESULTS. *Escherichia coli* ATCC 25922 -SITE 2

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	<1.0 x 10^1	> 5.28	99.9995
2	<1.0 x 10^1	> 5.28	99.9995
3	<1.0 x 10^1	> 5.28	99.9995

Soil Titer: 2.2 x 10^7 CFU/mL
Positive Device Titer: 1.91 x 10^5 CFU/site 2

TABLE 4: DISINFECTION RESULTS. *Escherichia coli* ATCC 25922 -SITE 3

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	<1.0 x 10^1	> 5.28	99.9995
2	<1.0 x 10^1	> 5.28	99.9995
3	<1.0 x 10^1	> 5.28	99.9995

Soil Titer: 2.2 x 10^7 CFU/mL
Positive Device Titer: 1.91 x 10^5 CFU/site 3

TABLE 5: DISINFECTION RESULTS. *Escherichia coli* ATCC 25922 -SITE 4

Test	CFU/mL	Log Reduction	Percent Reduction (%)
1	<1.0 x 10^1	> 5.32	99.9995
2	<1.0 x 10^1	> 5.32	99.9995
3	<1.0 x 10^1	> 5.32	99.9995

Soil Titer: 2.2 x 10^7 CFU/mL
Positive Device Titer: 2.07 x 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: *Escherichia coli* ATCC 25922

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	150	1.50×10^5
2	100.1	0.1	142	1.42×10^5
3	100.1	0.1	148	1.48×10^5
		Positive Control Titer:		1.47×10^5
		Log 10		5.17

Site 1

Organism: *Escherichia coli* ATCC 25922

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	$<1.0 \times 10^1$	> 5.17	>99.9993
2	100.0	<1	$<1.0 \times 10^1$	> 5.17	>99.9993
3	101.3	<1	$<1.0 \times 10^1$	> 5.17	>99.9993

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	38
CFU Plate 2:	45
CFU Plate 3:	49
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	2.2×10^7 CFU/mL

Positive control, **Site 2**

Organism: *Escherichia coli* ATCC 25922

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	193	1.93×10^5
2	100.3	0.1	183	1.83×10^5
3	100.3	0.1	199	1.99×10^5
		Positive Control Titer:		1.92×10^5
		Log 10		5.28

Site 2

Organism: *Escherichia coli* ATCC 25922

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	101.2	<1	$<1.0 \times 10^1$	> 5.28	> 99.9995
2	100.0	<1	$<1.0 \times 10^1$	> 5.28	> 99.9995
3	100.1	<1	$<1.0 \times 10^1$	> 5.28	> 99.9995

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	38
CFU Plate 2:	45
CFU Plate 3:	49
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	2.2×10^7 CFU/mL

Positive control, **Site 3**

Organism: *Escherichia coli* ATCC 25922

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	201	2.01×10^5
2	100.3	0.1	200	2.00×10^5
3	100.3	0.1	215	2.15×10^5
		Positive Control Titer:		2.05×10^5
		Log 10		5.31

Site 3

Organism: *Escherichia coli* ATCC 25922

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	103.2	<1	$<1.0 \times 10^1$	> 5.31	> 99.9995
2	101.0	<1	$<1.0 \times 10^1$	> 5.31	> 99.9995
3	101.1	<1	$<1.0 \times 10^1$	> 5.31	> 99.9995

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	38
CFU Plate 2:	45
CFU Plate 3:	49
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	2.2×10^7 CFU/mL

Positive control, **Site 4**

Organism: *Escherichia coli* ATCC 25922

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Volume IN (mL)	Diluent Plated (mL)	CFU/plate	CFU/mL
1	101.2	0.1	198	1.98×10^5
2	101.2	0.1	187	1.87×10^5
3	101.2	0.1	192	1.92×10^5
		Positive Control Titer:		1.92×10^5
		Log 10		5.28

Site 4

Organism: *Escherichia coli* ATCC 25922

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	101.2	<1	$<1.0 \times 10^1$	> 5.28	> 99.9995
2	100.2	<1	$<1.0 \times 10^1$	> 5.28	> 99.9995
3	100.1	<1	$<1.0 \times 10^1$	> 5.28	> 99.9995

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	38
CFU Plate 2:	45
CFU Plate 3:	49
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	2.2×10^7 CFU/mL

SPREAD PLATING DATA

Positive control, **Site 1**

Organism: *Escherichia coli* ATCC 25922

Number of Positives: 1

POSITIVE CONTROL, TITER CALCULATION

Replicate number	Volume IN (mL)	Dilution factor	CFU/plate	CFU/mL
1	0.1	1:100	165	1.65×10^5
2	0.1	1:100	159	1.59×10^5
3	0.1	1:100	162	1.62×10^5
		Positive Control Titer:		1.62×10^5
		Log 10		5.21

Site 1

Organism: *Escherichia coli* ATCC 25922

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	$<1.0 \times 10^1$	> 5.21	> 99.9994
2	0.1	10^0	<1	$<1.0 \times 10^1$	> 5.21	> 99.9994
3	0.1	10^0	<1	$<1.0 \times 10^1$	> 5.21	> 99.9994

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	38
CFU Plate 2:	45
CFU Plate 3:	49
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	2.2×10^7 CFU/mL

Positive control, **Site 2**

Organism: *Escherichia coli* ATCC 25922

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	189	1.89×10^5
2	0.1	1:100	199	1.99×10^5
3	0.1	1:100	185	1.85×10^5
		Positive Control Titer:		1.91×10^5
		Log 10		5.28

Site 2

Organism: *Escherichia coli* ATCC 25922

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	$<1.0 \times 10^1$	> 5.28	> 99.9995
2	0.1	10^0	<1	$<1.0 \times 10^1$	> 5.28	> 99.9995
3	0.1	10^0	<1	$<1.0 \times 10^1$	> 5.28	> 99.9995

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	38
CFU Plate 2:	45
CFU Plate 3:	49
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	2.2×10^7 CFU/mL

Positive control, **Site 3**

Organism: *Escherichia coli* ATCC 25922

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	197	1.97×10^5
2	0.1	1:100	190	1.90×10^5
3	0.1	1:100	187	1.87×10^5
		Positive Control Titer:		1.91×10^5
		Log 10		5.28

Site 3

Organism: *Escherichia coli* ATCC 25922

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	$<1.0 \times 10^1$	> 5.28	> 99.9995
2	0.1	10^0	<1	$<1.0 \times 10^1$	> 5.28	> 99.9995
3	0.1	10^0	<1	$<1.0 \times 10^1$	> 5.28	> 99.9995

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	38
CFU Plate 2:	45
CFU Plate 3:	49
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	2.2×10^7 CFU/mL

Positive control, **Site 4**

Organism: *Escherichia coli* ATCC 25922

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	2.10×10^5
2	0.1	1:100	199	1.99×10^5
3	0.1	1:100	213	2.13×10^5
		Positive Control Titer:		2.07×10^5
		Log 10		5.32

Site 4

Organism: *Escherichia coli* ATCC 25922

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	$<1.0 \times 10^1$	> 5.32	> 99.9995
2	0.1	10^0	<1	$<1.0 \times 10^1$	> 5.32	> 99.9995
3	0.1	10^0	<1	$<1.0 \times 10^1$	> 5.32	> 99.9995

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	38
CFU Plate 2:	45
CFU Plate 3:	49
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	2.2×10^7 CFU/mL

DIRECT PLATING DATA

Positive control, **Site 1**

Organism: *Escherichia coli* ATCC 25922

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	31	1.55×10^6
2	0.02	1:1000	33	1.65×10^6
3	0.02	1:1000	30	1.50×10^6
		Positive Control Titer:		1.57×10^6
		Log 10		6.20

Site 1

Organism: *Escherichia coli* ATCC 25922

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	$<1.0 \times 10^1$	> 6.20	> 99.9999
2	0.02	10^0	<1	$<1.0 \times 10^1$	> 6.20	> 99.9999
3	0.02	10^0	<1	$<1.0 \times 10^1$	> 6.20	> 99.9999

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	38
CFU Plate 2:	45
CFU Plate 3:	49
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	2.2×10^7 CFU/mL

Positive control, **Site 2**

Organism: *Escherichia coli* ATCC 25922

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	1.40×10^6
2	0.02	1:1000	35	1.75×10^6
3	0.02	1:1000	33	1.65×10^6
		Positive Control Titer:		1.6×10^6
		Log 10		6.20

Site 2

Organism: *Escherichia coli* ATCC 25922

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	$<1.0 \times 10^1$	> 6.20	> 99.9999
2	0.02	10^0	<1	$<1.0 \times 10^1$	> 6.20	> 99.9999
3	0.02	10^0	<1	$<1.0 \times 10^1$	> 6.20	> 99.9999

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	38
CFU Plate 2:	45
CFU Plate 3:	49
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	2.2×10^7 CFU/mL

Positive control, **Site 3**

Organism: *Escherichia coli* ATCC 25922

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	39	1.95×10^6
2	0.02	1:1000	35	1.75×10^6
3	0.02	1:1000	32	1.60×10^6
		Positive Control Titer:		1.77×10^6
		Log 10		6.25

Site 3

Organism: *Escherichia coli* ATCC 25922

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	$<1.0 \times 10^1$	> 6.25	> 99.9999
2	0.02	10^0	<1	$<1.0 \times 10^1$	> 6.25	> 99.9999
3	0.02	10^0	<1	$<1.0 \times 10^1$	> 6.25	> 99.9999

CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	38
CFU Plate 2:	45
CFU Plate 3:	49
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	2.2×10^7 CFU/mL

Positive control, **Site 4**

Organism: *Escherichia coli* ATCC 25922

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	31	1.55×10^6
2	0.02	1:1000	28	1.40×10^6
3	0.02	1:1000	32	1.60×10^6
		Positive Control Titer:		1.52×10^6
		Log 10		6.18

Site 4

Organism: *Escherichia coli* ATCC 25922

Number of devices tested: 1

Number of replicate tests: 3

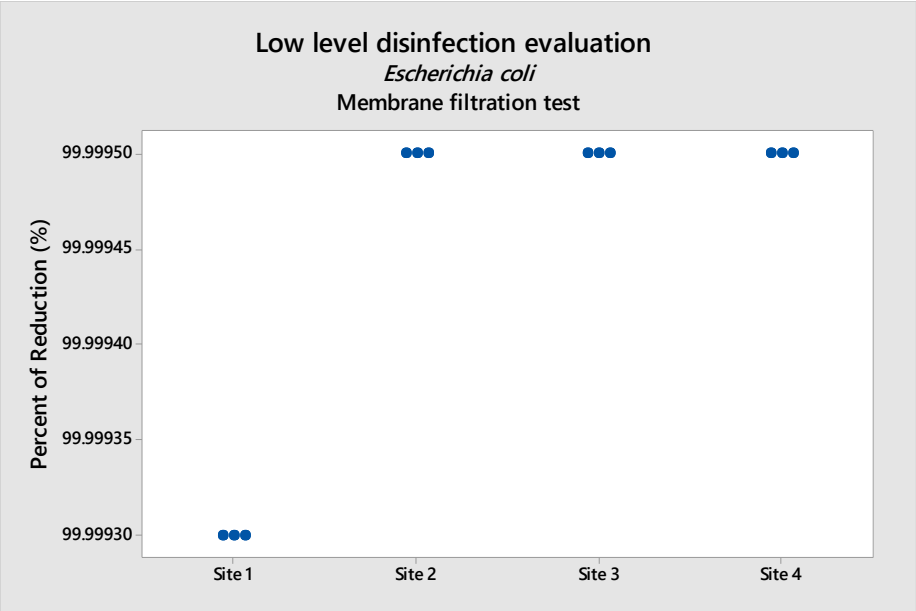
SITE 3 after 90-sec disinfection cycle

Device	Vol plated (mL)	Dilution Factor (mL)	CFU/plate	CFU/mL	Log Reduction	Percent Reduction (%)
1	0.02	10^1	<1	$<1.0 \times 10^1$	> 6.18	> 99.9999
2	0.02	10^1	<1	$<1.0 \times 10^1$	> 6.18	> 99.9999
3	0.02	10^1	<1	$<1.0 \times 10^1$	> 6.18	> 99.9999

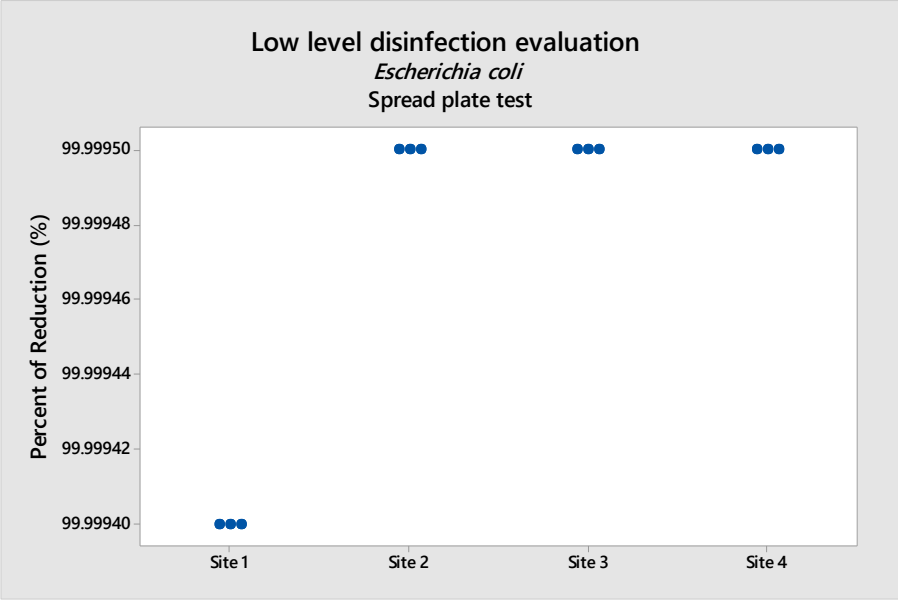
CFU= Colony forming unit
< = None detected

Inoculum	
CFU Plate 1:	38
CFU Plate 2:	45
CFU Plate 3:	49
Dilution:	10000
Volume plated (ml):	0.020
Initial CFU/mL:	2.2×10^7 CFU/mL

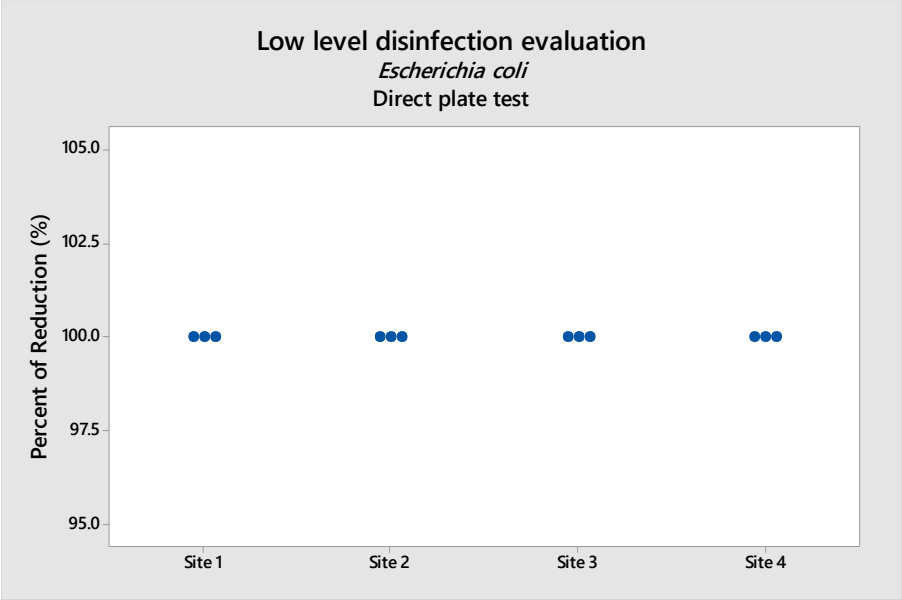
APPENDIX B: Graphics



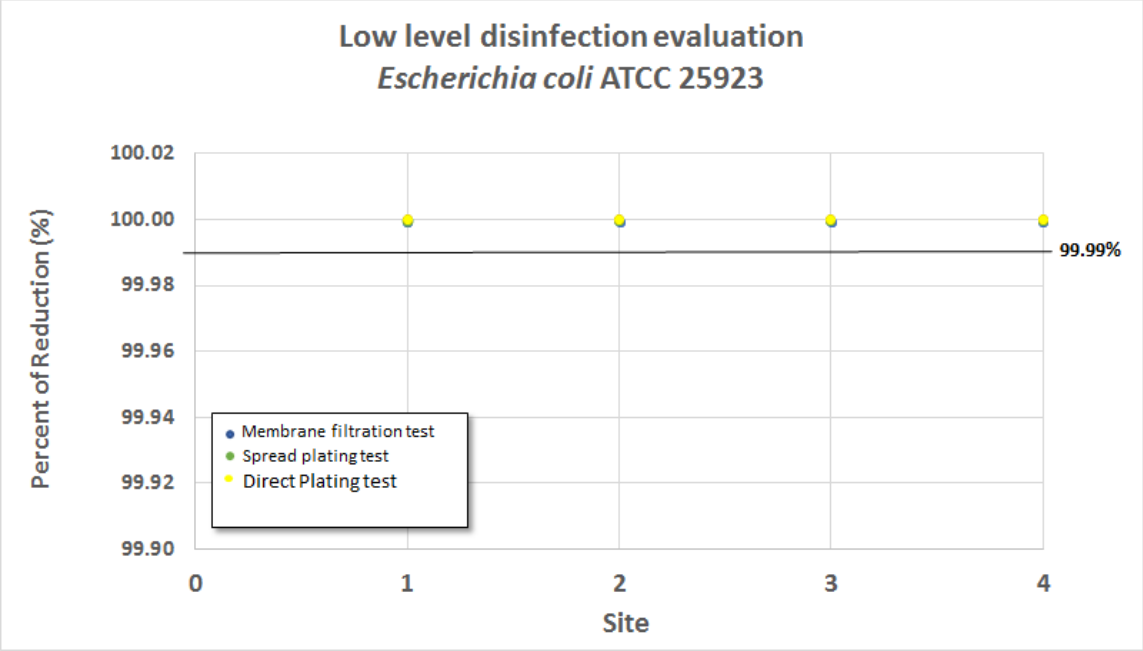
Graphic 1: Percent of reduction of low level disinfection evaluation of *Escherichia coli* ATCC 25922. Membrane filtration test.



Graphic 2: Percent of reduction of low level disinfection evaluation of *Escherichia coli* ATCC 25922. Spread Plating test.



Graphic 3: Percent of reduction of low level disinfection evaluation of *Escherichia coli* ATCC 25922. Direct Plating



Graphic 4: Percent of reduction of low level disinfection evaluation of *Escherichia coli* ATCC 25922

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: _____

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: 06/01/2013

Escherichia coli ATCC 25922				
10^{-3}	10^{-4}	10^{-5}	CFU/mL	Average
>	38	3		2.2×10^7
>	45	2		
>	49	3		

CONTROL RESULTS:
MEDIA EMB
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: 35°C TEMP 36 °C

DR 06/01/13

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:

☑ MEMBRANE FILTRATION
☑ DIRECT PLATING
☑ SPREAD PLATE: AMOUNT PLATED 0.1 mL

MEDIA

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

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LOW LEVEL DISINFECTION EVALUATION VALIDATION FORMS

Date: 05/30/2019

Organism(s)	E. coli		
ATCC #	25922		
Culture Age	18 hours		
Plate Media/Lot#	TSA Lot # 05/19		
Incubator ID#	0336	Time/Date In:	05/30/19 4:45 p.m
Temperature:	36.6°C	Time/Date Out:	05/31/19 10:15 a.m

Inoculum preparation	
Abs at 420 nm	0.154
Prepared in/Lot #	SI Bovine Serum
Spectrophotometer ID#	C0236 Thermo Scientific Genesys 10

Equipment:

	Ondine ID#	Calibration verified	Additional equipment ☒ N/A
Micropipettes	C0034, 0388, C0330, C0227	☑ Yes ☐ N/A	
Vortex	C0075, C0095	☐ 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	SY #1	☐ Yes ☑ N/A	

Supplies	LOT #	EXP /Manufacturer	Additional equipment ☐ N/A
Peptone-Tween water	0830E / 19035	Criterion Jan-20	- EMB Hasty-Be 05/2019 Criterion Lot #
Purified water	1610424	Fisher Oct-19	
Deionized water	VE C60410	Integra	- 1 mL Syringe BD
Bovine Serum	Cat Supplier #12007 19A85	Seradigm Jan-20	- 10 mL Syringe BD.
Sterile swabs	9913	Puritan Oct-20	
Filter	70053113	Pall	
Filter unit	N/A	Nalgene	
PBS	0519B212	Sigma-Aldrich	
TSB	5195Q	BD March-20	
Tween 80	0237119	Integra 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

E coli ATCC 25922

☞ 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.	0.1mL

☞ 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	98:20	20.6℃
	Site #2	45.13	
	Site #3	51.10	
	Site #4	43.13	
Test Device 1	Site #1	52.38	19.6℃
	Site #2	59.15	
	Site #3	47.12	
	Site #4	48.58	
Test Device 2	Site #1	43.18	20.4℃
	Site #2	49.03	
	Site #3	42.20	
	Site #4	45.23	
Test Device 3	Site #1	51.15	19.7℃
	Site #2	45.15	
	Site #3	89.08	
	Site #4	43.21	

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	41.3-46.3
Test Device 2	90 sec	40.1-46.0
Test Device 3	90 sec	39.1-45.3

☞ 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: 05/31/2013

Organism Used: E. coli ATCC 25922

Device	Vol. plated	SPREAD PLATE DILUTION						
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	CFU/mL
Positive Device Site #1	0.1 mL			165	3	0	0	1.65×10^3
	↓			159	5	0	0	1.59×10^3
	↓			162	8	0	0	1.62×10^3
Positive Device Site #2	0.1mL			189	5	0	0	1.89×10^3
	↓			199	8	0	0	1.99×10^3
	↓			185	10	1	0	1.85×10^3
Positive Device Site #3	0.1mL			193	10	1	0	1.93×10^3
	↓			190	15	0	0	1.90×10^3
	↓			187	13	0	0	1.87×10^3
Positive Device Site #4	0.1mL			210	21	3	0	2.1×10^3
	↓			199	28	1	0	1.99×10^3
	↓			213	19	0	0	2.13×10^3

Device	Vol in 1mL = 10	MEMBRANE FILTRATION		
		1.0 mL 0.1mL	10 mL	CFU/mL
Positive Device Site #1	100.1	160		$=1.60 \times 10^5$
		192		$=1.92 \times 10^5$
		148		$=1.48 \times 10^5$
Positive Device Site #2	100.3	193		$=1.93 \times 10^5$
		183		1.83×10^5
		199		1.99×10^5
Positive Device Site #3	100.3	201		2.01×10^5
		200		2×10^5
		215		2.15×10^5
Positive Device Site #4	101.2	198		1.9×10^5
		187		1.87×10^5
		192		1.92×10^5

Device	Vol. plated (mL)	DIRECT PLATING						
		10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	CFU/mL
Positive Device Site #1	0.02			31	6	0		$=1.53 \times 10^6$
				33	3	0		$=1.65 \times 10^6$
				30	4	1		1.50×10^6
Positive Device Site #2	0.02			28	5	0		1.40×10^6
				35	7	1		1.75×10^6
				33	2	0		1.65×10^6
Positive Device Site #3	0.02			37	6	0		$=1.85 \times 10^6$
				35	1	0		$=1.75 \times 10^6$
				32	3	0		$=1.60 \times 10^6$
Positive Device Site #4	0.02			31	5	1		$=1.40 \times 10^6$
				28	2	0		$=1.40 \times 10^6$
				32	3	0		$=1.52 \times 10^6$

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LOW LEVEL DISINFECTION EVALUATION
VALIDATION FORMS

TEST DEVICE 1

Date: 05/31/2019 - 06/02/19
Organism Used: E. coli 25922

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

Device	Vol in mL	MEMBRANE FILTRATION 1.0 mL	10 mL	CFU/mL
TD1 Site #1	100.1	0	0	0
		0	↓	
		0		
TD1 Site #2	100.1	0	0	0
		0	↓	
		0		
TD1 Site #3	101.3	0	0	0
		0	↓	
		0		
TD1 Site #4	99.9	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
		0	0	0				
		0	0	0				
TD1 Site #2	0.02	0	0	0				=0
		0	0	0				
		0	0	0				
TD1 Site #3	0.02	0	0	0				=0
		0	0	0				
		0	0	0				
TD1 Site #4	0.07	0	0	0				=0
		0	0	0				
		0	0	0				

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LOW LEVEL DISINFECTION EVALUATION
VALIDATION FORMS

TEST DEVICE 2

Date: 05/13/2013 - 06/07/13

Organism Used: E. coli ATCC 25922

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
	↓	0	0	0				
		0	0	0				

Device	Vol in mL	MEMBRANE FILTRATION		
		1.0 mL	10 mL	CFU/mL
TD2 Site #1	100.1	0	0	0
		↓	↓	
TD2 Site #2	100.8	0	0	0
		↓	↓	
TD2 Site #3	102.0	0	0	0
		↓	↓	
TD2 Site #4	100.0	0	0	0
		↓	↓	

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				
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: 05/31/2017 - 06/02/17
Organism Used: E. coli

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					
TD3 Site #2	0.1 mL	0	0	0				0
		0	0	0				
		0	0	0				
TD3 Site #3	0.1 mL	0	0	0				0
		0	0	0				
		0	0	0				
TD3 Site #4	0.1 mL	0	0	0				0
		0	0	0				
		0	0	0				

Device	Vol in 1g = 1mL	MEMBRANE FILTRATION		
		1.0 mL	10 mL	CFU/mL
TD3 Site #1	101.3	0	0	0
		0	0	
		0	0	
TD3 Site #2	103.1	0	0	0
		0	0	
		0	0	
TD3 Site #3	103.2	0	0	0
		0	0	
		0	0	
TD3 Site #4	101.4	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
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				

END OF DOCUMENT