



NSF International – Applied Research Center
789 N. Dixboro Rd. Ann Arbor, MI 48015, USA
1-800.NSF.MARK | +1-734.769.8010 | www.nsf.org

Send to: Scott Beal
Hepco Medical, LLC
200 Central Ave Ste 2200
St. Petersburg, FL 33701

Result: COMPLETE

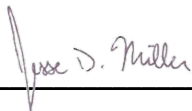
Report Date: August 23, 2019

Customer Name: Hepco Medical, LLC
Description: Efficacy of an Ozone-Generating Whole-Shoe Disinfection Device at Three Time Points
Test Type: Test Only
Job Number: J-00340388
Project Number: 10120011
NSF Corporate: C0484938
Project Manager: S. Hatt

Executive Summary: An efficacy study was performed using a UV-C and ozone-generating device against *Escherichia coli*, *Pseudomonas aeruginosa*, Methicillin-resistant *Staphylococcus aureus*, Vancomycin-resistant *Enterococcus faecalis*, Carbapenem-resistant *Klebsiella pneumoniae*, *Candida auris*, *Aspergillus brasiliensis*, and *Clostridioides difficile*. Log and percent reduction were quantified for each microorganism at three exposure times: 6, 8, and 10 seconds.

Thank you for having your product tested by NSF International.

Please contact your Project Manager if you have any questions or concerns pertaining to this report.

Report Authorization: 
Jesse Miller – Director, Applied Research Center

Experimental Summary:

Challenge microorganisms:

- *Escherichia coli* ATCC 11229
- *Pseudomonas aeruginosa* ATCC BAA- 2108
- Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33592
- Vancomycin-resistant *Enterococcus faecalis* (VRE) ATCC 51299
- Carbapenem-resistant *Klebsiella pneumoniae* (CRE) ATCC BAA-1705
- *Candida auris* CDC B11903
- *Aspergillus brasiliensis* ATCC 16404
- *Clostridioides difficile* ATCC 43598

Test Product:

- PathO₃Gen Solutions™ Footwear Sanitizing Station

Culture Preparation:

- Methicillin-resistant *Staphylococcus aureus* (MRSA), Vancomycin-resistant *Enterococcus faecalis* (VRE), *Klebsiella pneumoniae* (CRE), and *Escherichia coli* were propagated onto Tryptic Soy Agar with 5% Sheep Blood (SBA) and were incubated at 35 ± 2°C for 24 ± 2 hours.
- *Candida auris* was propagated onto SBA for 18-24 hours at 25 ± 1°C.
 - Daily transfers were performed using Sabouraud Dextrose Agar with Letheen (SDA/L). Each daily transfer was incubated at the appropriate temperature for growth for 24 ± 2 hours.
 - After incubation, an isolated colony was picked to SDA/L and incubated at 35 ± 2°C for 24 ± 2 hours.
- *Aspergillus brasiliensis* was propagated on SDA/L for 5 to 7 days. After incubation, the culture was washed with 0.85% saline with tween and filtered with through sterile gauze. The culture was centrifuged at 4,500 rpm, the supernatant removed, and the pellet was rehydrated with Phosphate Buffered Saline (PBS).
- *Clostridium difficile* spore suspension was prepared using a modification of the U.S. EPA OPP: MB-28 (December 2017) Procedure for the Production and Storage of Spores of *Clostridium difficile* for Use in the Efficacy Evaluation of Antimicrobial Agents based on ASTM Standard E2839-11.

Inoculation:

- Hard rubber carriers of approximately 2" x 2" were sterilized prior to testing.
- The soles of the shoe carriers were inoculated with 0.1 mL aliquot of standardized suspension of the challenge microorganism and allowed to dry for 60 ± 5 minutes.

Exposure Period:

- The disinfection device was sterilized using isopropyl alcohol prior to testing.
- After drying, the sole of the shoe carrier was aseptically placed inoculum side down onto the floor disinfection device using sterilized forceps.
- A volunteer (~150 lb) stood on the shoe carrier (with a sterile barrier between the individual and shoe carrier) for the exposure time. The instrument automatically shut off after the exposure time.
- After the exposure time, the shoe carrier was moved to a saline solution using sterile forceps. Three carriers were tested per each microorganism.
 - For bacteria, dilutions were plated via pour plate method in duplicate on Microbial Content Test Agar and incubated for 48 ± 3 hours at 35 ± 2 °C
 - For fungi, dilutions were plated via pour plate method in duplicate on Sabouraud Dextrose Agar with Letheen and incubated for 5 to 7 days at 25 ± 2 °C
 - For spores, dilutions were plated via spread plate method in duplicate on Brucella Blood Agar and incubated for 48 ± 3 hours at 36 ± 2 °C.
- After incubation, colonies were counted, and data recorded. Geometric mean was calculated from the duplicated plates and log and percent reduction were calculated using the positive control counts.

Results

TEST REPORT

Table 1. Geometric mean of the inoculum concentrations on unexposed carriers used for each microorganism. The results shown are the geomean of the inoculum plated in triplicate.

Organism	Time Point	CFU/mL	Log (CFU/mL)
E. coli ATCC 11229	6 seconds	3.03E+07	7.4814
	8 seconds	59000000	7.7706
	10 seconds	3.26E+07	7.5129
Pseudomonas aeruginosa ATCC BAA- 2108	6 seconds	1.52E+06	6.1829
	8 seconds	2.21E+06	6.3439
	10 seconds	1.60E+06	6.2037
Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA) ATCC 33592	6 seconds	2.79E+07	7.4461
	8 seconds	4.36E+07	7.6398
	10 seconds	4.01E+07	7.6030
Vancomycin-resistant <i>Enterococcus faecalis</i> (VRE) ATCC 51299	6 seconds	7.12E+07	7.8528
	8 seconds	7.40E+07	7.8690
	10 seconds	4.64E+07	7.6664
<i>Klebsiella pneumoniae</i> CRE ATCC BAA-1705	6 seconds	1.97E+08	8.2944
	8 seconds	3.36E+08	8.5262
	10 seconds	2.69E+08	8.4302
<i>Candida auris</i> CDC B11903	6 seconds	5.16E+06	6.7129
	8 seconds	1.32E+06	6.1219
	10 seconds	1.86E+06	6.2692
<i>Aspergillus brasiliensis</i> ATCC 16404	6 seconds	6.70E+06	6.8261
	8 seconds	8.41E+06	6.9246
	10 seconds	1.39E+07	7.1438
<i>Clostridioides difficile</i> ATCC 43598	6 seconds	1.15E+07	7.0614
	8 seconds	1.37E+07	7.1380
	10 seconds	1.24E+07	7.0927

Table 2. Carrier density for each of the carriers inoculated with *E. coli* and exposed to the disinfection device. The results shown are the geomean of each of the carriers, which were plated in triplicate.

Organism	Time Point	Replicate A (CFU/mL)	Replicate B (CFU/mL)	Replicate C (CFU/mL)	Geomean (CFU/mL)	Log CFU/mL	Percent Reduction	Log Reduction
<i>E. coli</i> ATCC 11229	6 seconds	3.96E+05	3.59E+05	1.29E+05	2.64E+05	5.4211	99.1297%	2.06
	8 seconds	2.95E+04	4.14E+04	3.51E+03	1.62E+04	4.2107	99.9725%	3.56
	10 seconds	1.23E+02	4.11E+02	9.23E+02	3.60E+02	2.5563	99.9989%	4.96

Table 3. Carrier density for each of the carriers inoculated with *Pseudomonas aeruginosa* and exposed to the disinfection device. The results shown are the geomean of each of the carriers, which were plated in triplicate.

Organism	Time Point	Replicate A (CFU/mL)	Replicate B (CFU/mL)	Replicate C (CFU/mL)	Geomean (CFU/mL)	Log CFU/mL	Percent Reduction	Log Reduction
<i>Pseudomonas aeruginosa</i> ATCC BAA- 2108	6 seconds	1.89E+03	2.84E+03	3.29E+03	2.60E+03	3.4157	99.8287%	2.77
	8 seconds	4.32E+01	5.15E+01	6.53E+01	5.26E+01	1.7207	99.9976%	4.62
	10 seconds	5.90E+01	6.39E+01	2.82E+01	4.74E+01	1.6755	99.9970%	4.53

Table 4. Carrier density for each of the carriers inoculated with MRSA and exposed to the disinfection device. The results shown are the geomean of each of the carriers, which were plated in triplicate.

Organism	Time Point	Replicate A (CFU/mL)	Replicate B (CFU/mL)	Replicate C (CFU/mL)	Geomean (CFU/mL)	Log CFU/mL	Percent Reduction	Log Reduction
Methicillin Resistant <i>Staphylococcus aureus</i> (MRSA) ATCC 33592	6 seconds	2.73E+04	5.25E+04	3.75E+04	3.77E+04	4.5768	99.8647%	2.87
	8 seconds	4.90E+03	5.78E+03	1.15E+04	6.88E+03	3.8376	99.9842%	3.80
	10 seconds	5.18E+02	1.15E+03	3.30E+03	1.25E+03	3.0978	99.9969%	4.51

Table 5. Carrier density for each of the carriers inoculated with VRE and exposed to the disinfection device. The results shown are the geomean of each of the carriers, which were plated in triplicate.

Organism	Time Point	Replicate A (CFU/mL)	Replicate B (CFU/mL)	Replicate C (CFU/mL)	Geomean (CFU/mL)	Log CFU/mL	Percent Reduction	Log Reduction
<i>Enterococcus faecalis</i> VRE ATCC 51299	6 seconds	1.08E+06	1.16E+06	8.14E+05	1.01E+06	6.0028	98.5863%	1.85
	8 seconds	5.83E+03	1.28E+04	1.29E+04	9.87E+03	3.9945	99.9867%	3.87
	10 seconds	7.62E+03	5.88E+03	5.77E+03	6.37E+03	3.8042	99.9863%	3.86

Table 6. Carrier density for each of the carriers inoculated with CRE and exposed to the disinfection device. The results shown are the geomean of each of the carriers, which were plated in triplicate.

Organism	Time Point	Replicate A (CFU/mL)	Replicate B (CFU/mL)	Replicate C (CFU/mL)	Geomean (CFU/mL)	Log CFU/mL	Percent Reduction	Log Reduction
<i>Klebsiella pneumoniae</i> CRE ATCC BAA-1705	6 seconds	7.26E+05	3.86E+05	1.29E+06	7.12E+05	5.8527	99.6384%	2.44
	8 seconds	8.75E+04	1.20E+05	2.72E+05	1.42E+05	5.1519	99.9578%	3.37
	10 seconds	3.91E+03	1.32E+03	9.85E+03	3.70E+03	3.5687	99.9986%	4.86

Table 7. Carrier density for each of the carriers inoculated with *Candida auris* and exposed to the disinfection device. The results shown are the geomean of each of the carriers, which were plated in triplicate. For plate count geomeans below 10 CFU/mL were input as 10 to calculate percent and log reduction.

Organism	Time Point	Replicate A (CFU/mL)	Replicate B (CFU/mL)	Replicate C (CFU/mL)	Geomean (CFU/mL)	Log CFU/mL	Percent Reduction	Log Reduction
<i>Candida auris</i> CDC B11903	6 seconds	1.09E+03	1.82E+02	6.08E+02	4.94E+02	2.6938	99.9904%	4.02
	8 seconds	1.46E+02	<1.00E+01	2.93E+01	3.50E+01	1.5437	99.9974%	4.58
	10 seconds	2.11E+01	<1.00E+01	<1.00E+01	1.28E+01	1.1081	99.9993%	5.16

Table 8. Carrier density for each of the carriers inoculated with *Aspergillus brasiliensis* and exposed to the disinfection device. The results shown are the geomean of each of the carriers, which were plated in triplicate.

Organism	Time Point	Replicate A (CFU/mL)	Replicate B (CFU/mL)	Replicate C (CFU/mL)	Geomean (CFU/mL)	Log CFU/mL	Percent Reduction	Log Reduction
<i>Aspergillus brasiliensis</i> ATCC 16404	6 seconds	1.18E+06	1.13E+06	1.08E+06	1.13E+06	6.0528	83.1453%	0.77
	8 seconds	5.29E+05	1.00E+06	1.16E+06	8.50E+05	5.9293	89.8956%	1.00
	10 seconds	3.69E+05	1.25E+06	3.26E+05	5.32E+05	5.7257	96.1744%	1.42

Table 9. Carrier density for each of the carriers inoculated with *C. difficile* and exposed to the disinfection device. The results shown are the geomean of each of the carriers, which were plated in triplicate.

Organism	Time Point	Replicate A (CFU/mL)	Replicate B (CFU/mL)	Replicate C (CFU/mL)	Geomean (CFU/mL)	Log CFU/mL	Percent Reduction	Log Reduction
<i>Clostridioides difficile</i> ATCC 43598	6 seconds	1.21E+05	8.61E+04	1.87E+05	1.25E+05	5.0965	98.9140%	1.96
	8 seconds	9.65E+03	9.62E+03	4.86E+03	7.67E+03	3.8848	99.9440%	3.25
	10 seconds	2.26E+03	1.47E+03	3.63E+01	4.94E+02	2.6938	99.9960%	4.40

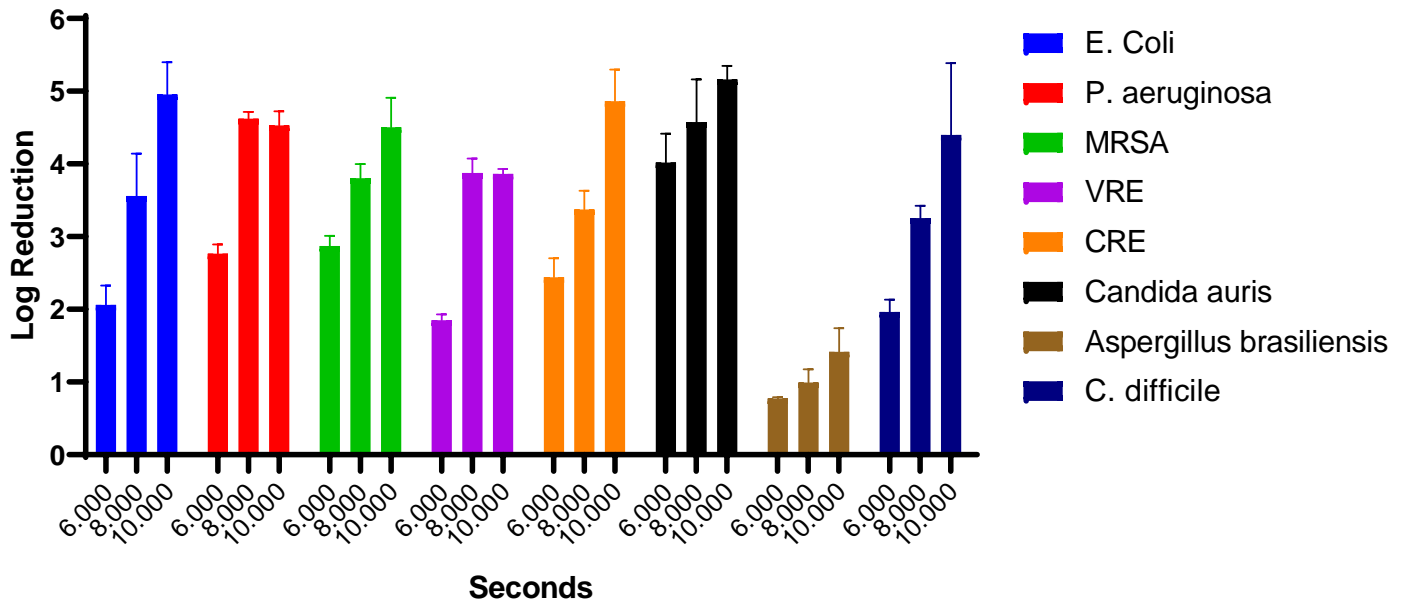


Figure 1. Summary bar plot of mean log reduction at each time point (in seconds) by microorganism tested.

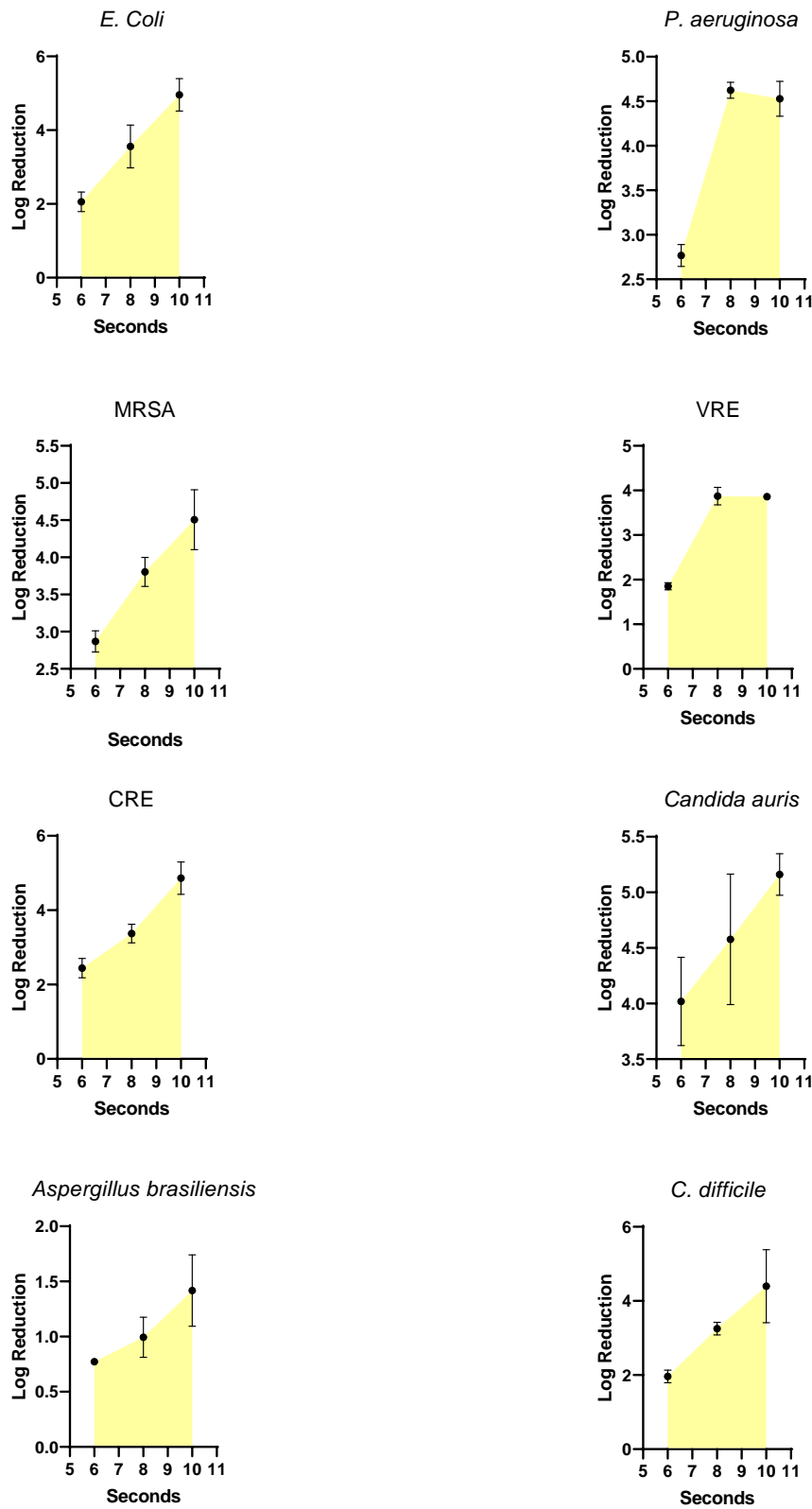


Figure 2. Individual point plots of the mean log reduction at each time point for each microorganism tested.



NSF International – Applied Research Center
 789 N. Dixboro Rd. Ann Arbor, MI 48015, USA
 1-800.NSF.MARK | +1-734.769.8010 | www.nsf.org

Table 10. Linear regression analysis results assessing the relationship of log reduction by time. The slopes for each line are significantly different from zero.

	<i>E. Coli</i>	<i>P. aeruginosa</i>	MRSA	VRE	CRE	<i>Candida auris</i>	<i>Aspergillus brasiliensis</i>	<i>C. difficile</i>
Best-fit values								
Slope	0.7242	0.4403	0.409	0.5031	0.605	0.2854	0.1612	0.6087
Y-intercept	-2.268	0.4502	0.4534	-0.8288	-1.281	2.303	-0.2276	-1.664
X-intercept	3.132	-1.023	-1.109	1.647	2.117	-8.067	1.412	2.734
1/slope	1.381	2.271	2.445	1.988	1.653	3.503	6.204	1.643
Std. Error								
Slope	0.08503	0.1099	0.05277	0.1138	0.06902	0.0799	0.04195	0.111
Y-intercept	0.6942	0.8973	0.4309	0.929	0.5635	0.6524	0.3425	0.9065
95% Confidence Intervals								
Slope	0.5231 to 0.9253	0.1804 to 0.7002	0.2842 to 0.5338	0.2340 to 0.7721	0.4418 to 0.7682	0.09649 to 0.4744	0.06200 to 0.2604	0.3462 to 0.8712
Y-intercept	-3.910 to -0.6265	-1.672 to 2.572	-0.5654 to 1.472	-3.025 to 1.368	-2.613 to 0.05183	0.7600 to 3.845	-1.038 to 0.5822	-3.808 to 0.4792
X-intercept	1.187 to 4.264	-14.10 to 2.413	-5.151 to 1.065	-5.766 to 3.972	-0.1165 to 3.427	-39.58 to -1.613	-9.251 to 4.045	-1.366 to 4.428
Goodness of Fit								
R square	0.912	0.6963	0.8956	0.7364	0.9165	0.6458	0.6784	0.8111
Sy.x	0.4165	0.5384	0.2585	0.5574	0.3381	0.3914	0.2055	0.5439
Is slope significantly non-zero?								
F	72.54	16.05	60.08	19.55	76.83	12.76	14.77	30.06
DFn, DFd	1, 7	1, 7	1, 7	1, 7	1, 7	1, 7	1, 7	1, 7
P value	<0.0001	0.0051	0.0001	0.0031	<0.0001	0.0091	0.0064	0.0009
Deviation from zero?	Significant	Significant	Significant	Significant	Significant	Significant	Significant	Significant
Equation	Y = 0.7242*X - 2.268	Y = 0.4403*X + 0.4502	Y = 0.4090*X + 0.4534	Y = 0.5031*X - 0.8288	Y = 0.6050*X - 1.281	Y = 0.2854*X + 2.303	Y = 0.1612*X - 0.2276	Y = 0.6087*X - 1.664

J-00340388

Page 9 of 11

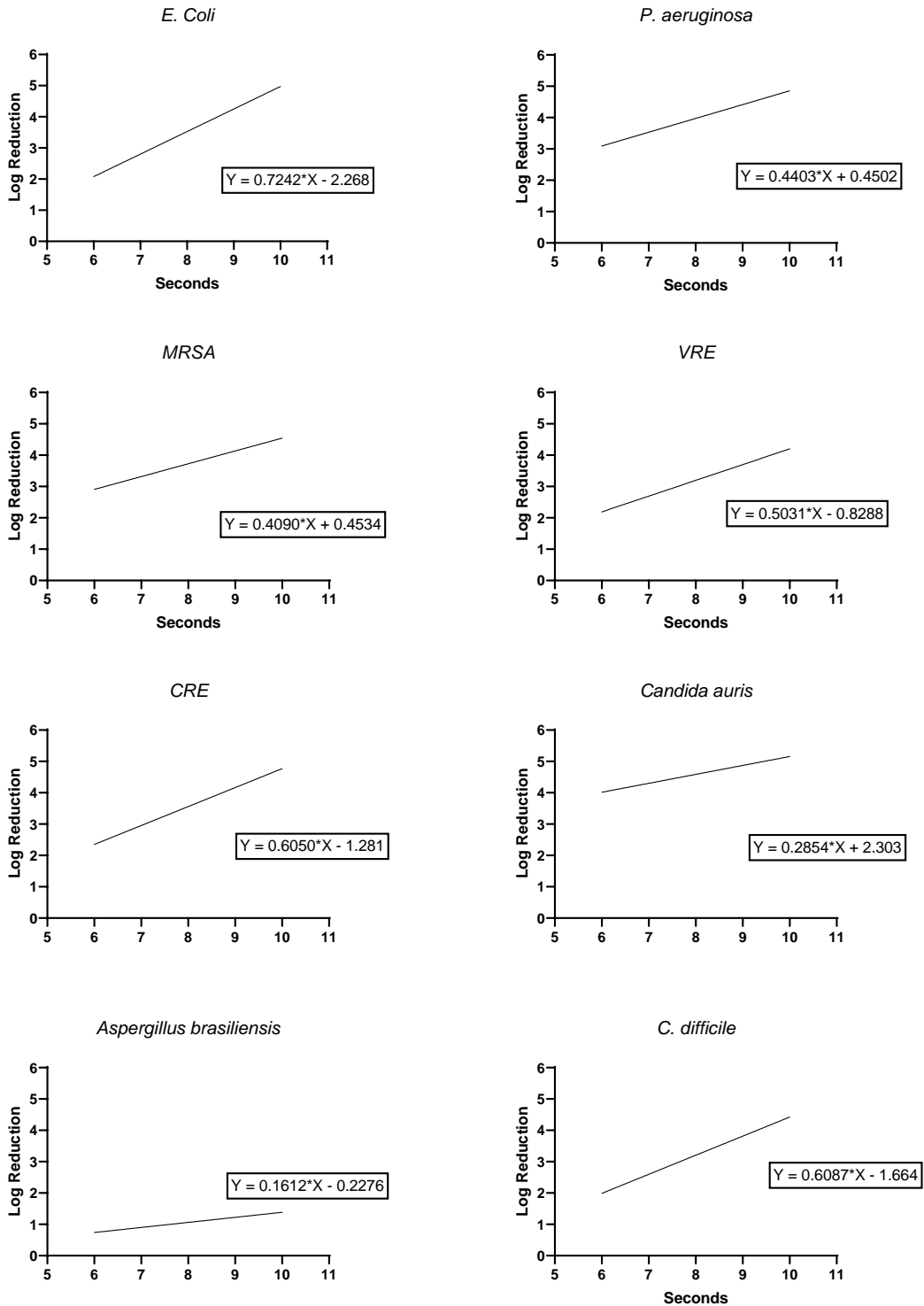


Figure 3. Regression analysis plot for each microorganism. Formula for the line is presented for each plot.

Testing Laboratories:

All work performed at:

Lab ID
Approved Subcontract

Note
GLP, non-GLP compliant