

Suppressing *E. coli* Biofilm Formation with Garlic Extract and  
Justifying Conclusions with the Philosophy of Science

Appendices

Christopher Skalnik

Global Scholars Project

Park Tudor School

Indianapolis, IN

Mentor: Dr. Gregory Anderson

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## Appendix A: Raw Data

The tables below present the raw measurements collected during the two trials, along with some calculations. The five replicates are displayed in columns, followed by the mean, the 95% confidence interval, the standard deviation, and the standard error of the mean that are calculated from those five replicates. Note that only the standard error of the mean (SEM) and the mean are displayed in the graphs. The experimental and vehicle control samples are grouped under a corresponding label, and the row headers indicate the concentrations of ethanol or garlic extract added.

Table 7: Trial 1 Raw Data

Raw Data	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	95% Conf.	Standard Deviation	Standard Error of the Mean
Negative Control	0.036	0.023	0.015	0.015	0.023	0.022	0.0107	0.0086	0.0038
Positive Control	0.079	0.076	0.071	0.072	0.071	0.074	0.0044	0.0036	0.0016
Experimental ( $\mu\text{L} / \text{mL}$ )									
2.0	0.071	0.049	0.048	0.046	0.042	0.051	0.0141	0.0114	0.0051
8.0	0.121	0.091	0.061	0.054	0.051	0.076	0.0372	0.0299	0.0134
16	0.233	0.143	0.150	0.116	0.095	0.147	0.0654	0.0526	0.0235
64	0.026	0.025	0.027	0.032	0.028	0.028	0.0034	0.0027	0.0012
Vehicle Control ( $\mu\text{L} / \text{mL}$ )									
2.0	0.076	0.069	0.062	0.056	0.040	0.061	0.0171	0.0137	0.0061
8.0	0.070	0.068	0.079	0.060	0.047	0.065	0.0149	0.0120	0.0054
16	0.129	0.096	0.042	0.039	0.040	0.069	0.0512	0.0412	0.0184
64	0.029	0.040	0.049	0.049	0.042	0.042	0.0102	0.0082	0.0037

**Table 8: Trial 2 Raw Data**

Raw Data	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	95% Conf.	Standard Deviation	Standard Error of the Mean
Negative Control	0.019	0.020	0.019	0.018	0.016	0.018	0.0019	0.0015	0.0007
Positive Control	0.115	0.136	0.136	0.163	0.119	0.134	0.0235	0.0189	0.0085
Experimental ( $\mu\text{L} / \text{mL}$ )									
2.0	0.065	0.057	0.051	0.058	0.058	0.058	0.0062	0.0050	0.0022
8.0	0.060	0.064	0.064	0.060	0.053	0.060	0.0056	0.0045	0.0020
16	0.156	0.092	0.112	0.103	0.105	0.114	0.0307	0.0248	0.0111
64	0.033	0.024	0.025	0.020	0.026	0.026	0.0059	0.0047	0.0021
Vehicle Control ( $\mu\text{L} / \text{mL}$ )									
2.0	0.182	0.133	0.108	0.055	0.055	0.107	0.0672	0.0541	0.0242
8.0	0.067	0.068	0.078	0.085	0.070	0.074	0.0096	0.0077	0.0034
16	0.054	0.045	0.039	0.047	0.045	0.046	0.0067	0.0054	0.0024
64	0.040	0.061	0.051	0.047	0.061	0.052	0.0113	0.0091	0.0041

**Table 9: Trial 3 Raw Data**

Raw Data	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	95% Conf.	Standard Deviation	Standard Error of the Mean
Negative Control	0.018	0.019	0.019	0.017	0.019	0.018	0.0011	0.0009	0.0004
Positive Control	0.039	0.037	0.030	0.040	0.032	0.036	0.0055	0.0044	0.0020
Experimental ( $\mu\text{L} / \text{mL}$ )									
2.0	0.041	0.035	0.033	0.031	0.032	0.034	0.0049	0.0040	0.0018
8.0	0.074	0.056	0.048	0.035	0.040	0.051	0.0190	0.0153	0.0069
16	0.160	0.106	0.084	0.083	0.067	0.100	0.0451	0.0363	0.0162
64	0.025	0.028	0.034	0.032	0.032	0.030	0.0045	0.0036	0.0016
Vehicle Control ( $\mu\text{L} / \text{mL}$ )									
2.0	0.265	0.300	0.319	0.346	0.314	0.309	0.0368	0.0296	0.0132
8.0	0.198	0.202	0.219	0.251	0.241	0.222	0.0290	0.0234	0.0105
16	0.176	0.195	0.212	0.213	0.213	0.202	0.0203	0.0163	0.0073
64	0.022	0.026	0.026	0.028	0.026	0.026	0.0027	0.0022	0.0010

**Table 10: Trial 4 Raw Data**

Raw Data	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	95% Conf.	Standard Deviation	Standard Error of the Mean
Negative Control	0.059	0.069	0.051	0.027	0.030	0.047	0.0227	0.0183	0.0082
Positive Control	0.121	0.119	0.122	0.091	0.100	0.111	0.0176	0.0142	0.0063
Experimental ( $\mu\text{L} / \text{mL}$ )									
2.0	0.058	0.068	0.078	0.078	0.069	0.070	0.0103	0.0083	0.0037
8.0	0.058	0.060	0.076	0.072	0.075	0.068	0.0106	0.0086	0.0038
16	0.095	0.085	0.081	0.076	0.083	0.084	0.0087	0.0070	0.0031
64	0.032	0.051	0.038	0.033	0.038	0.038	0.0094	0.0076	0.0034
Vehicle Control ( $\mu\text{L} / \text{mL}$ )									
2.0	0.061	0.052	0.057	0.068	0.075	0.063	0.0113	0.0091	0.0041
8.0	0.073	0.075	0.078	0.080	0.061	0.073	0.0092	0.0074	0.0033
16	0.080	0.079	0.082	0.081	0.078	0.080	0.0020	0.0016	0.0007
64	0.059	0.038	0.065	0.101	0.079	0.068	0.0291	0.0234	0.0105

**Table 11: Trial 5 Raw Data**

Raw Data	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 5	Mean	95% Conf.	Standard Deviation	Standard Error of the Mean
Negative Control	0.033	0.019	0.020	0.021	0.026	0.024	0.0072	0.0058	0.0026
Positive Control	0.217	0.206	0.238	0.206	0.341	0.242	0.0709	0.0571	0.0255
Experimental ( $\mu\text{L} / \text{mL}$ )									
2.0	0.062	0.108	0.083	0.080	0.175	0.102	0.0549	0.0442	0.0198
8.0	0.072	0.070	0.066	0.118	0.191	0.103	0.0662	0.0534	0.0239
16	0.081	0.039	0.040	0.035	0.039	0.047	0.0239	0.0192	0.0086
64	0.060	0.058	0.155	0.112	0.270	0.131	0.1087	0.0875	0.0391
Vehicle Control ( $\mu\text{L} / \text{mL}$ )									
2.0	0.066	0.051	0.055	0.062	0.064	0.060	0.0079	0.0063	0.0028
8.0	0.086	0.051	0.053	0.066	0.069	0.065	0.0175	0.0141	0.0063
16	0.068	0.074	0.072	0.073	0.059	0.069	0.0076	0.0061	0.0027
64	0.033	0.025	0.026	0.023	0.025	0.026	0.0048	0.0038	0.0017

**Table 12: Raw Data for Combining All Trials**

Raw Data ( $\mu\text{L}$ / mL)	Exp 16 Raw Mean	Exp 18 Raw Mean	Exp 19 Raw Mean	Exp 20 Raw Mean	Exp 21 Raw Mean	Exp 16 Norm. Mean	Exp 18 Norm. Mean	Exp 19 Norm. Mean	Exp 20 Norm. Mean	Exp 21 Norm. Mean	Mean	95% Conf.	Standard Deviation	Standard Error of Mean
Negative Control	0.022	0.018	0.0184	0.0472	0.0238	0.000	0.000	0.000	0.000	0.000	0.0000	0.0000	0.0000	0.0000
Positive Control	0.074	0.134	0.0356	0.1106	0.2416	0.696	0.862	0.129	0.573	0.901	0.565	0.7807	0.3143	0.1815
Experimental														
2	0.051	0.058	0.0344	0.0702	0.1016	0.390	0.294	0.120	0.208	0.322	0.253	0.2882	0.1160	0.0670
8	0.076	0.060	0.0506	0.0682	0.1034	0.721	0.312	0.241	0.190	0.329	0.366	0.6009	0.2419	0.1397
16	0.147	0.114	0.1	0.084	0.0468	1.694	0.712	0.610	0.333	0.095	0.837	1.4736	0.5932	0.3425
64	0.028	0.026	0.0302	0.0384	0.131	0.070	0.054	0.088	-0.080	0.444	0.033	0.1900	0.0765	0.0442
Vehicle Control														
2	0.061	0.107	0.3088	0.0626	0.0596	0.518	0.659	2.170	0.139	0.148	0.872	2.2189	0.8932	0.5157
8	0.065	0.074	0.2222	0.0734	0.065	0.575	0.413	1.523	0.237	0.171	0.687	1.4268	0.5744	0.3316
16	0.069	0.046	0.2018	0.08	0.0692	0.634	0.206	1.371	0.297	0.188	0.627	1.3140	0.5289	0.3054
64	0.042	0.052	0.0256	0.0684	0.0264	0.263	0.401	0.043	0.192	0.011	0.225	0.3700	0.1489	0.0860

The table above presents, in the first five columns, the average values for each sample from the five trials conducted. The next five columns contain the normalized forms of the first five. Normalization was achieved by subtracting the values from the negative control and expressing the result as a fraction of the positive control readings.

## **Appendix B: Figures**

The figures below document both the growth and the staining visible in the tubes. The cultures were photographed after 24 hours of growth, and the staining was photographed after drying overnight. In Figures 1, 3, 5, 7, and 9, the green arrow indicates how clarity increases with concentration among the experimental samples, and the red arrow indicates the same trend among the vehicle controls. This reflects a decrease in bacterial growth, likely due to the 95% ethanol. In Figures 2, 4, 6, 8, and 10, the tubes in the green box generally have stronger staining than those in the red box. This indicates that at 16  $\mu\text{L} / \text{mL}$ , the experimental samples generally show more biofilm formation than the associated vehicle controls and suggest garlic extract's promotion of biofilm formation.

## Trial 1

Figure 1: Trial 1 Cultures

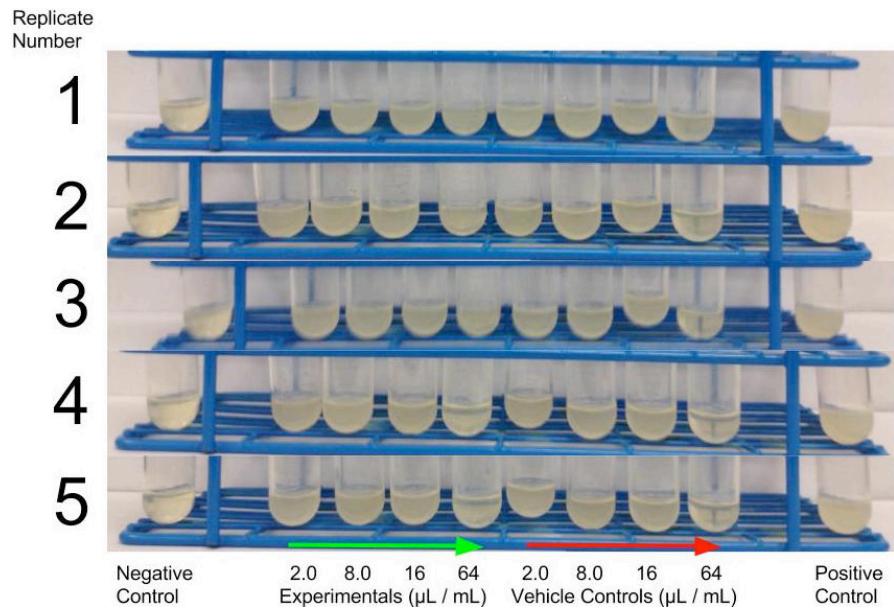
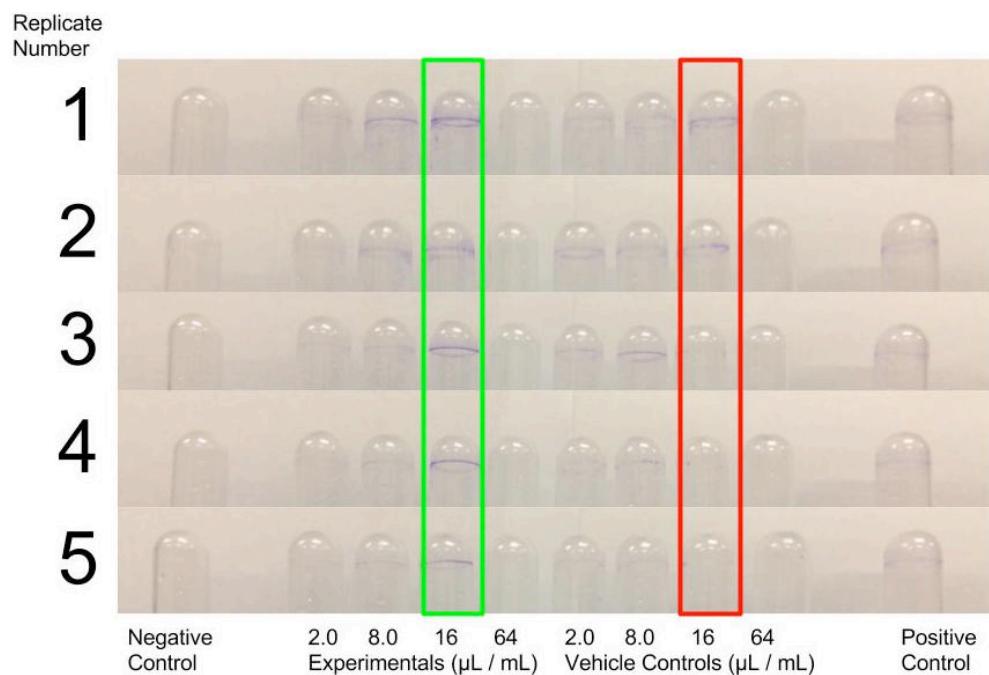


Figure 2: Trial 1 Staining



## Trial 2

Figure 3: Trial 2 Cultures

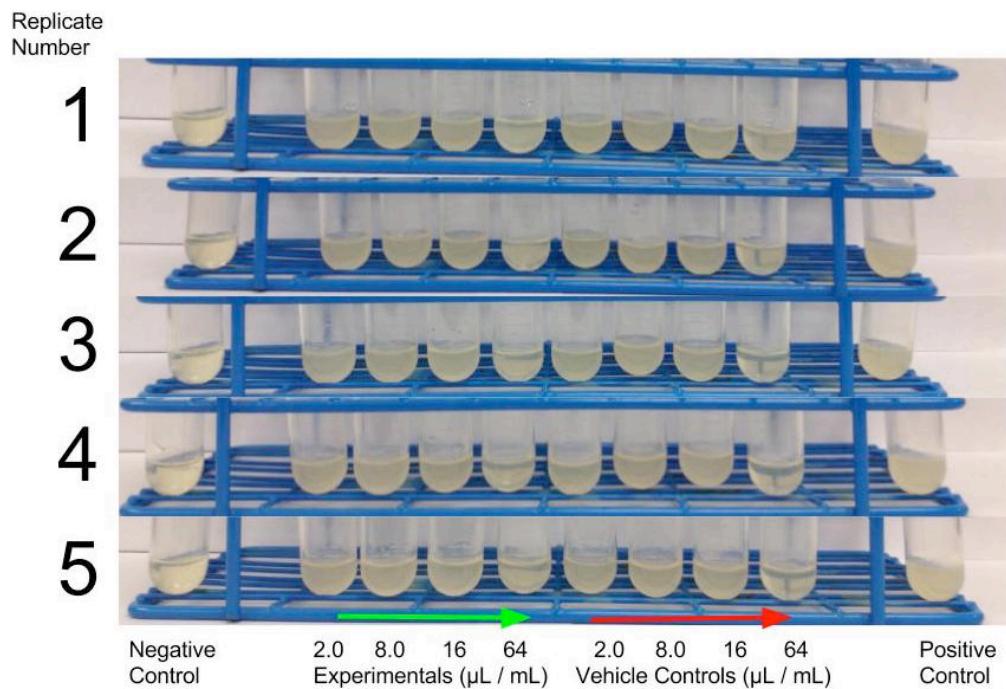
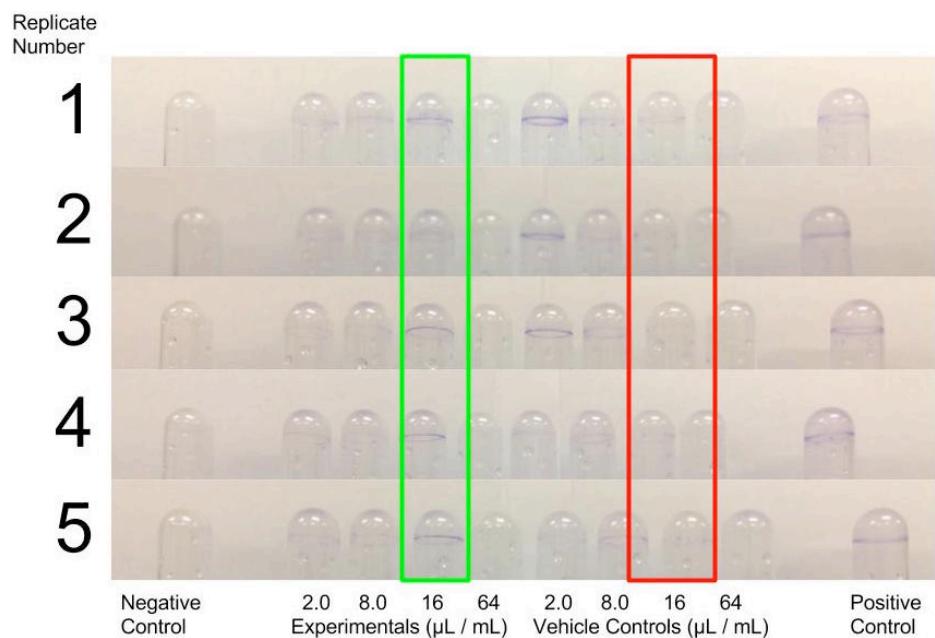


Figure 4: Trial 2 Staining



### Trial 3

Figure 5: Trial 3 Cultures

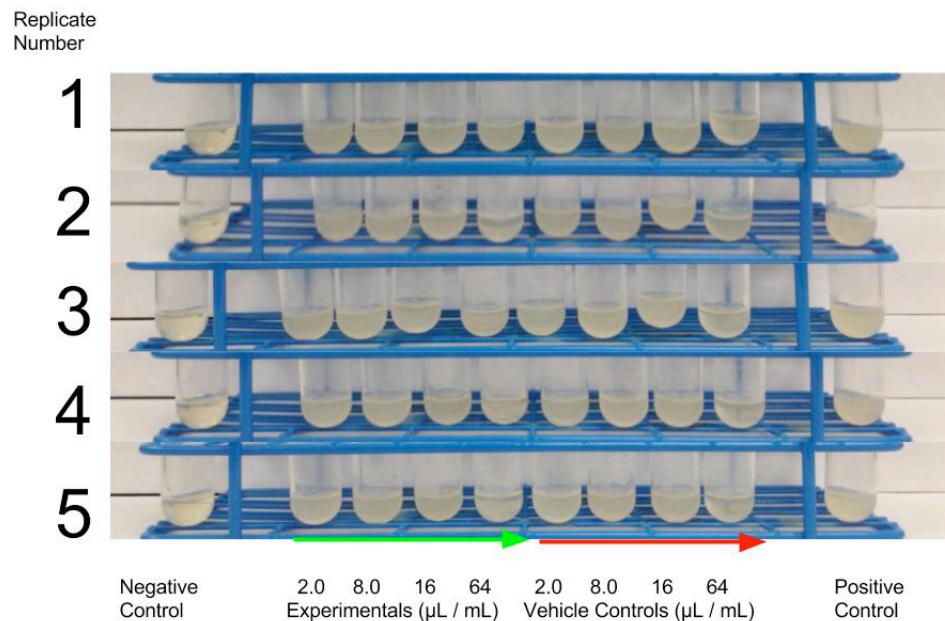
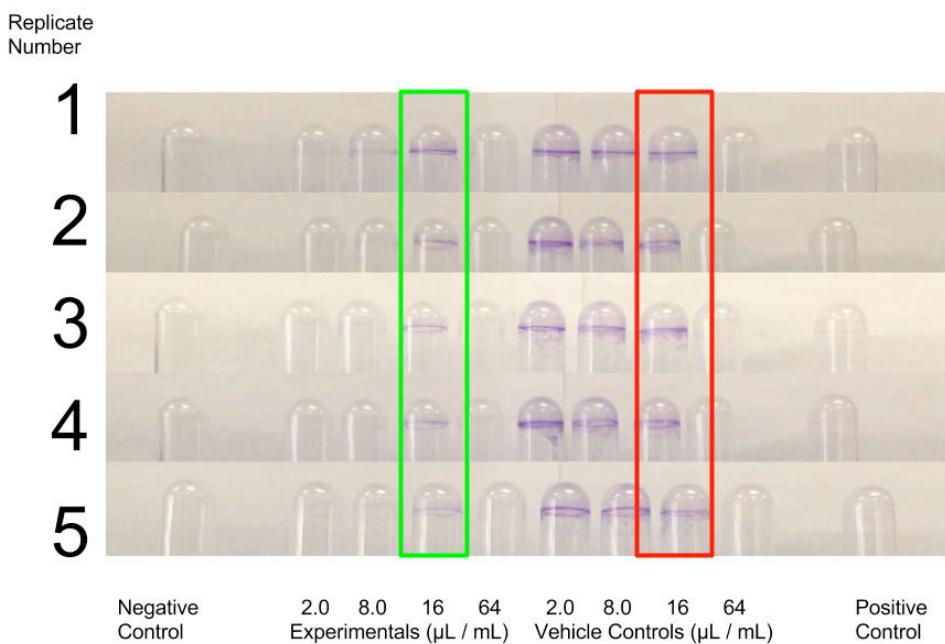


Figure 6: Trial 3 Staining



## Trial 4

Figure 7: Trial 4 Cultures

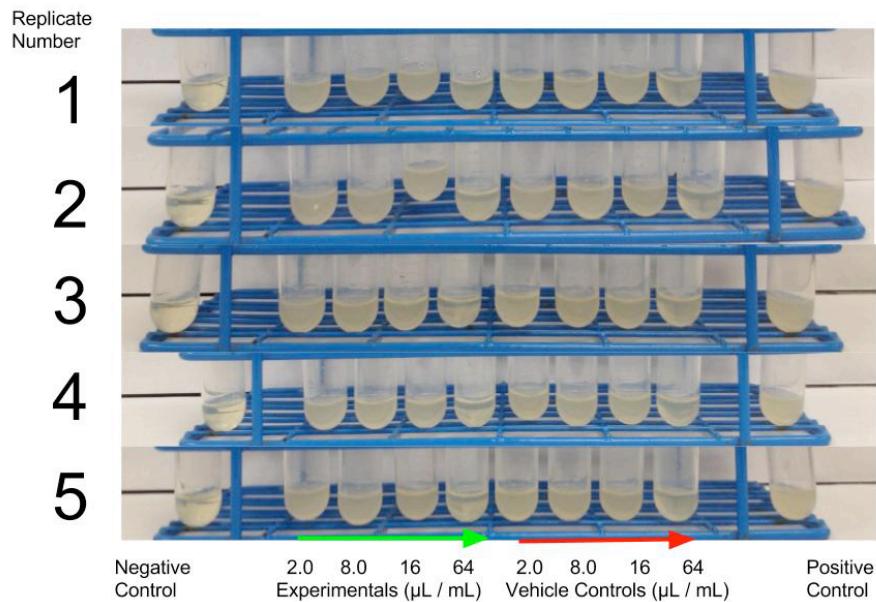
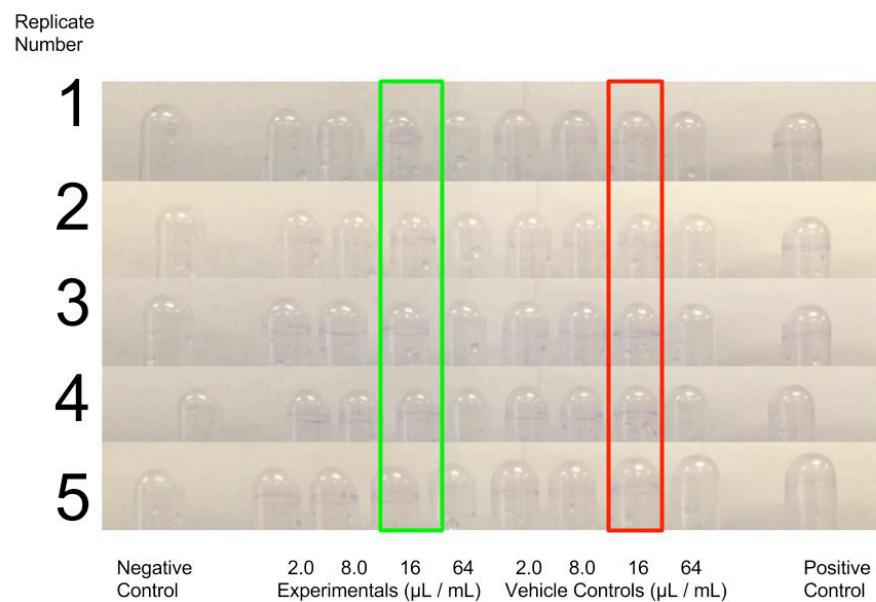


Figure 8: Trial 4 Staining



## Trial 5

Figure 9: Trial 5 Cultures

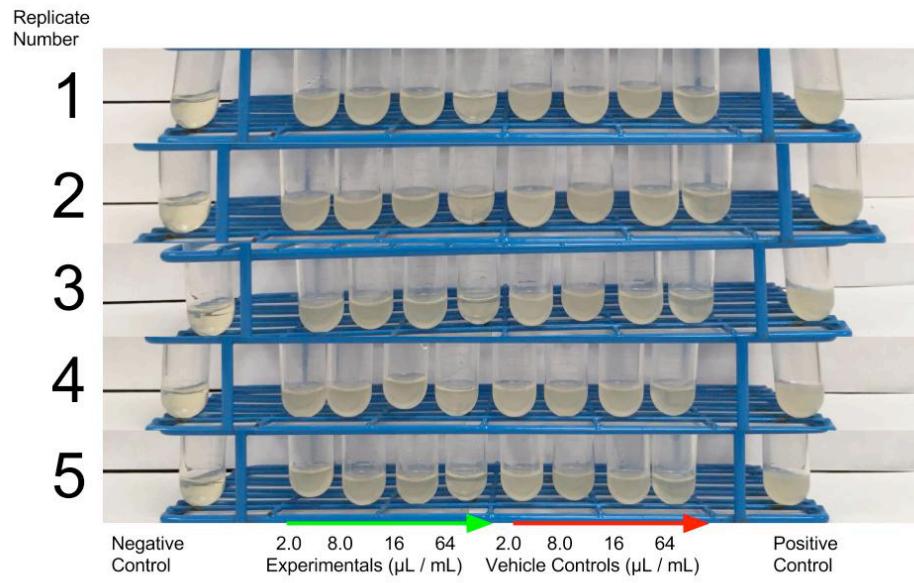


Figure 10: Trial 5 Staining

