

June 5, 2019

Beirut, Lebanon

To Entotox Public Health SARL,

Please find below the full report regarding ezoSafe by ezoMED water based disinfectant. Chemical characterization and microbiological assessment of a dodecyl dimethyl benzyl ammonium chloride / Copper π Complex under the name ezoSafe by ezoMED were performed. The microbiological assessments were performed using CLSI guidelines.

1- Using broth microdilution (BMD) technique, the range and spectrum of activity of ezoSafe was determined. A wide range of Gram-positive and Gram-negative pathogenic bacteria were tested in addition to *Candida albicans*. In brief, a serial dilution of the disinfectant of interest is applied horizontally in 96-well plate in a 100 μ L of cation adjusted Muller-Hinton broth (MHCAB). Subsequently, 10 μ L of the bacteria of interest is added at a concentration of 0.5 McFarland in each well. Total volume is then adjusted with additional MHCAB to reach a total of 200 μ L and the plates were incubated at 37 °C for 18 hours while shaking at 160 rpm.

2-Using BMD technique, test this compound against 300 various resistant microorganisms. A set of 100 clinical isolates of *Staphylococcus aureus* with various resistance profiles were tested. In addition a set of 100 clinical isolates of *Acinetobacter baumannii* and a set of 100 clinical isolates of *Pseudomonas aeruginosa* were also tested. In brief, a serial dilution of the drug of interest is applied horizontally in 96-well plate in a 100 μ L of cation adjusted Muller-Hinton broth (MHCAB). Subsequently, 10 μ L of the bacteria of interest is added at a concentration of 0.5 McFarland in each well. Total volume is then adjusted with additional MHCAB to reach a total of 200 μ L and the plates were incubated at 37 °C for 18 hours while shaking at 160 rpm.

3-Dilution factor was also performed in order to check at what percentage the disinfectant will still be active.

4- Time kill assay to determine the killing time and potential of the detergent, in addition to Log killing efficacy and Log reduction potential.

- MIC = Minimum Inhibitory Concentration, i.e. the lowest concentration of Product that prevents visible growth in a serial dilution array.
- 0.5 McFarland is equivalent to 1.5×10^8 CFU in every single well of the 96-well plate.

For further clarifications, please do not hesitate to contact us.

Yours sincerely,



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Table 1: MIC of ezoSafe against a variety of microorganisms.

Microorganisms	ezoSafe MIC (µg/mL)	Quaternary Control MIC (µg/mL)	Chlorhexidine MIC (µg/mL)
<i>S. aureus</i> ATCC 29213	0.0125	0.025	0.1
<i>S. aureus</i> Newman	0.0125	0.05	0.5
<i>S. aureus</i> N315	0.0125	0.05	0.5
<i>E. faecalis</i> ATCC 19433	0.0125	0.025	1
<i>E. faecium</i> VRE DSM 17050	0.025	0.2	2
<i>S. pneumoniae</i> ATCC 49619	0.0125	0.1	2
<i>A.baumannii</i> DSM 30008	0.025	0.1	2
<i>K. pneumoniae</i> ATCC 13883	0.025	0.1	2
<i>P. aeruginosa</i> ATCC 27853	0.1	0.5	2
<i>P. aeruginosa</i> mexAB	0.0125	0.05	0.5
<i>E. coli</i> ATCC 25922	0.025	0.05	0.1
<i>Candida albicans</i>	0.0125	0.05	0.5

Table 2: MIC of ezoSafe against 100 *Staphylococcus aureus* clinical isolates (from patients and hospitals) with various resistance profiles. Each “# (M.B)” is a clinical isolate with a unique genetic profile different from any other in the table below

# (M.B.)	MIC (µg/mL)		
	ezoSafe	Quaternary Control	Chlorhexidine
1	0.025	0.05	0.5
2	0.025	0.025	4
3	0.025	0.05	1
4	0.0125	0.025	2
5	0.025	0.025	1
6	0.1	0.05	2



7	0.05	0.025	2
8	0.05	0.05	1
9	0.1	0.05	2
10	0.0125	0.025	2
11	0.1	0.05	2
12	0.025	0.05	1
13	0.2	0.05	1
14	0.2	0.1	2
15	0.025	0.025	2
16	0.1	0.05	2
17	0.025	0.025	1
18	0.025	0.05	1
19	0.1	0.05	2
20	0.05	0.05	2
21	0.1	0.05	4
22	0.2	0.1	2
23	0.4	0.05	2
24	0.05	0.05	2
25	0.2	0.05	2
26	0.1	0.05	1
27	0.4	0.05	2
28	0.1	0.05	1
29	0.4	0.05	2
30	0.2	0.05	2
31	0.1	0.05	1
32	0.4	0.025	1
33	0.05	0.05	1
34	0.025	0.05	0.5
35	0.025	0.025	2
36	0.05	0.05	1
37	0.05	0.05	1
38	0.1	0.1	1
39	0.1	0.05	2
40	0.05	0.05	1
41	0.1	0.05	2
42	0.025	0.025	2
43	0.025	0.025	2
44	0.05	0.05	2
45	0.1	0.05	1
46	0.025	0.025	0.5
47	0.05	0.05	1
48	0.0125	0.05	2



49	0.1	0.05	2
50	0.025	0.05	2
51	0.1	0.05	1
52	0.1	0.05	1
53	0.1	0.05	2
54	0.1	0.0125	1
55	0.05	0.025	2
56	0.05	0.05	4
57	0.1	0.05	1
58	0.1	0.05	2
59	0.1	0.05	1
60	0.1	0.05	1
61	0.1	0.05	1
62	0.1	0.05	2
63	0.05	0.025	2
64	0.025	0.05	1
65	0.05	0.025	1
66	0.025	0.025	1
67	0.0125	0.025	1
68	0.05	0.05	1
69	0.05	0.05	4
70	0.05	0.025	1
71	0.05	0.05	2
72	0.0125	0.025	0.5
73	0.025	0.05	1
74	0.025	0.025	1
75	0.05	0.025	4
76	0.0125	0.025	1
77	0.025	0.0125	0.5
78	0.025	0.025	4
79	0.0125	0.0125	1
80	0.05	0.025	2
81	0.05	0.05	1
82	0.1	0.05	2
83	0.05	0.05	1
84	0.025	0.025	4
85	0.05	0.05	1
86	0.025	0.025	1
87	0.1	0.025	1
1a	0.0125	nd	0.5
2a	0.025	nd	1
3a	0.05	nd	0.5

4a	0.025	nd	0.5
5a	0.025	nd	1
6a	0.025	nd	2
7a	0.0125	nd	0.5
8a	0.05	nd	0.5
81a	0.2	0.025	1
82a	0.1	0.05	0.5
83a	0.2	0.025	1
84a	0.1	0.0125	1
85a	0.05	0.0125	1
86a	0.05	0.0125	1
87a	0.025	0.0125	1
88	0.1	0.025	2
89	0.2	0.025	1
90	0.4	0.0125	0.5
91	0.4	0.025	0.5
92	0.2	0.025	1
93	0.05	0.025	1
94	0.05	0.025	0.5
95	0.1	0.025	1
96	0.2	0.025	0.5
97	0.05	0.0125	0.5
98	0.1	0.025	0.5
99	0.05	0.025	1
100	0.1	0.025	1

Table 3: MIC of ezoSafe against 100 *Pseudomonas aeruginosa* clinical isolates (from patients and hospitals) with various resistance profiles. Each “# (M.B)” is a clinical isolate with a unique genetic profile different from any other in the table below.

# (M.B.)	MIC (µg/mL)		
	ezoSafe	Quaternary Control	Chlorhexidine
1	0.025	0.05	0.5
2	0.025	0.025	4
3	0.025	0.05	1
4	0.0125	0.025	2
5	0.025	0.025	1
6	0.1	0.05	2
7	0.05	0.025	2



8	0.05	0.05	1
9	0.1	0.05	2
10	0.0125	0.025	2
11	0.1	0.05	2
12	0.025	0.05	1
13	0.2	0.05	1
14	0.2	0.1	2
15	0.025	0.025	2
16	0.0125	0.1	2
17	0.025	0.025	1
18	0.025	0.05	1
19	0.0125	0.1	2
20	0.05	0.05	2
21	0.1	0.05	4
22	0.2	0.1	2
23	0.4	0.05	2
24	0.05	0.05	2
25	0.0125	0.1	2
26	0.0125	0.1	2
27	0.0125	0.1	2
28	0.1	0.05	1
29	0.0125	0.1	2
30	0.2	0.05	2
31	0.1	0.05	1
32	0.4	0.025	1
33	0.05	0.05	1
34	0.0125	0.1	2
35	0.025	0.025	2
36	0.05	0.05	1
37	0.05	0.05	1
38	0.1	0.1	1
39	0.1	0.05	2
40	0.05	0.05	1
41	0.1	0.05	2
42	0.025	0.025	2
43	0.025	0.025	2
44	0.05	0.05	2
45	0.1	0.05	1
46	0.025	0.025	0.5
47	0.05	0.05	1
48	0.0125	0.05	2
49	0.1	0.05	2



50	0.01	0.5	1
51	0.1	0.05	1
52	0.1	0.05	1
53	0.0125	0.05	4
54	0.0125	0.1	2
55	0.05	0.025	2
56	0.05	0.05	4
57	0.1	0.05	1
58	0.1	0.05	2
59	0.1	0.05	1
60	0.1	0.05	1
61	0.1	0.05	1
62	0.1	0.05	2
63	0.0125	0.1	2
64	0.025	0.05	1
65	0.05	0.025	1
66	0.025	0.025	1
67	0.0125	0.025	1
68	0.05	0.05	1
69	0.05	0.05	4
70	0.05	0.025	1
71	0.0125	0.1	2
72	0.0125	0.025	0.5
73	0.025	0.05	1
74	0.025	0.025	1
75	0.05	0.025	4
76	0.0125	0.025	1
77	0.025	0.0125	0.5
78	0.025	0.025	4
79	0.0125	0.0125	1
80	0.05	0.025	2
81	0.05	0.05	1
82	0.0125	0.1	2
83	0.05	0.05	1
84	0.025	0.025	4
85	0.05	0.05	1
86	0.025	0.025	1
87	0.0125	0.1	2
1a	0.0125	nd	0.5
2a	0.0125	0.1	2
3a	0.05	nd	0.5
4a	0.0125	0.1	2

5a	0.025	nd	1
6a	0.025	nd	2
7a	0.0125	nd	0.5
8a	0.0125	0.1	2
81a	0.2	0.025	1
82a	0.1	0.05	0.5
83a	0.2	0.025	1
84a	0.1	0.0125	1
85a	0.05	0.0125	1
86a	0.0125	0.1	2
87a	0.025	0.0125	1
88	0.1	0.025	2
89	0.2	0.025	1
90	0.4	0.0125	0.5
91	0.0125	0.1	2
92	0.2	0.025	1
93	0.05	0.025	1
94	0.05	0.025	0.5
95	0.1	0.025	1
96	0.2	0.025	0.5
97	0.0125	0.1	2
98	0.1	0.025	0.5
99	0.05	0.025	1
100	0.0125	0.1	2

Table 4: MIC of ezoSafe against 100 *Acinetobacter baumannii* clinical isolates (from patients and hospitals) with various resistance profiles. Each “ # (M.B)” is a clinical isolate with a unique genetic profile different from any other in the table below.

# (M.B.)	MIC (µg/mL)		
	ezoSafe	Quaternary Control	Chlorhexidine
1	0.025	0.05	0.5
2	0.025	0.025	4
3	0.025	0.05	1
4	0.0125	0.025	2
5	0.025	0.025	1
6	0.1	0.05	2
7	0.05	0.025	2
8	0.05	0.05	1
9	0.1	0.05	2
10	0.0125	0.025	2



11	0.1	0.05	2
12	0.025	0.05	1
13	0.2	0.05	1
14	0.2	0.1	2
15	0.025	0.025	2
16	0.0125	0.1	2
17	0.025	0.025	1
18	0.025	0.05	1
19	0.0125	0.1	2
20	0.05	0.05	2
21	0.1	0.05	4
22	0.2	0.1	2
23	0.4	0.05	2
24	0.05	0.05	2
25	0.0125	0.1	2
26	0.0125	0.1	2
27	0.0125	0.1	2
28	0.1	0.05	1
29	0.0125	0.1	2
30	0.2	0.05	2
31	0.1	0.05	1
32	0.05	0.05	1
33	0.05	0.05	1
34	0.0125	0.1	2
35	0.025	0.025	2
36	0.05	0.05	1
37	0.05	0.05	1
38	0.1	0.1	1
39	0.1	0.05	2
40	0.05	0.05	1
41	0.1	0.05	2
42	0.025	0.025	2
43	0.025	0.025	2
44	0.05	0.05	2
45	0.1	0.05	1
46	0.025	0.025	0.5
47	0.05	0.05	1
48	0.0125	0.05	2
49	0.1	0.05	2
50	0.01	0.5	1
51	0.1	0.05	1
52	0.1	0.05	1



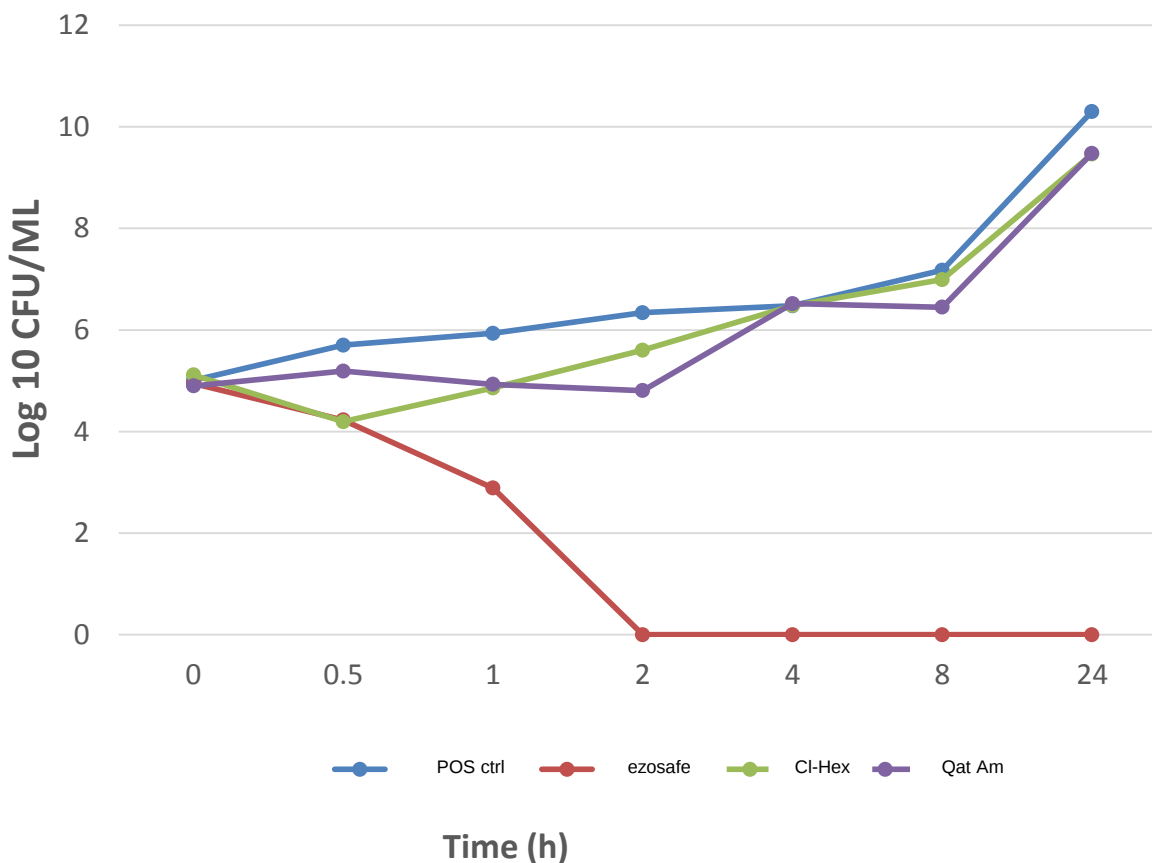
53	0.0125	0.05	4
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55	0.05	0.025	2
56	0.05	0.05	4
57	0.1	0.05	1
58	0.1	0.05	2
59	0.1	nd	1
60	0.1	0.05	1
61	0.1	0.05	1
62	0.1	0.05	2
63	0.0125	0.1	2
64	0.025	0.05	1
65	0.05	0.025	1
66	0.025	0.025	1
67	0.05	0.05	1
68	0.05	0.05	1
69	0.05	0.05	4
70	0.05	0.05	1
71	0.0125	0.1	2
72	0.0125	0.025	0.5
73	0.025	0.05	1
74	0.025	0.025	1
75	0.05	0.025	4
76	0.0125	0.025	1
77	0.025	0.0125	0.5
78	0.025	0.025	4
79	0.0125	0.0125	1
80	0.05	0.025	2
81	0.05	0.05	1
82	0.0125	0.1	2
83	0.05	0.05	1
84	0.025	0.025	4
85	0.05	0.05	1
86	0.025	0.025	1
87	0.0125	0.1	2
1a	0.0125	nd	0.5
2a	0.0125	nd	2
3a	0.05	nd	0.5
4a	0.0125	0.1	2
5a	0.025	nd	1
6a	0.025	nd	2
7a	0.0125	nd	0.5



8a	0.0125	0.1	2
81a	0.2	0.025	1
82a	0.1	0.05	0.5
83a	0.2	0.025	1
84a	0.1	nd	1
85a	0.05	0.0125	1
86a	0.0125	0.1	2
87a	0.025	0.0125	1
88	0.1	nd	2
89	0.2	0.025	1
90	0.4	0.0125	0.5
91	0.0125	0.1	2
92	0.05	0.05	1
93	0.05	0.025	1
94	0.05	0.025	0.5
95	0.1	0.025	1
96	0.05	n.d	1
97	0.0125	0.1	2
98	0.1	0.025	0.5
99	0.05	0.025	1
100	0.0125	0.1	2

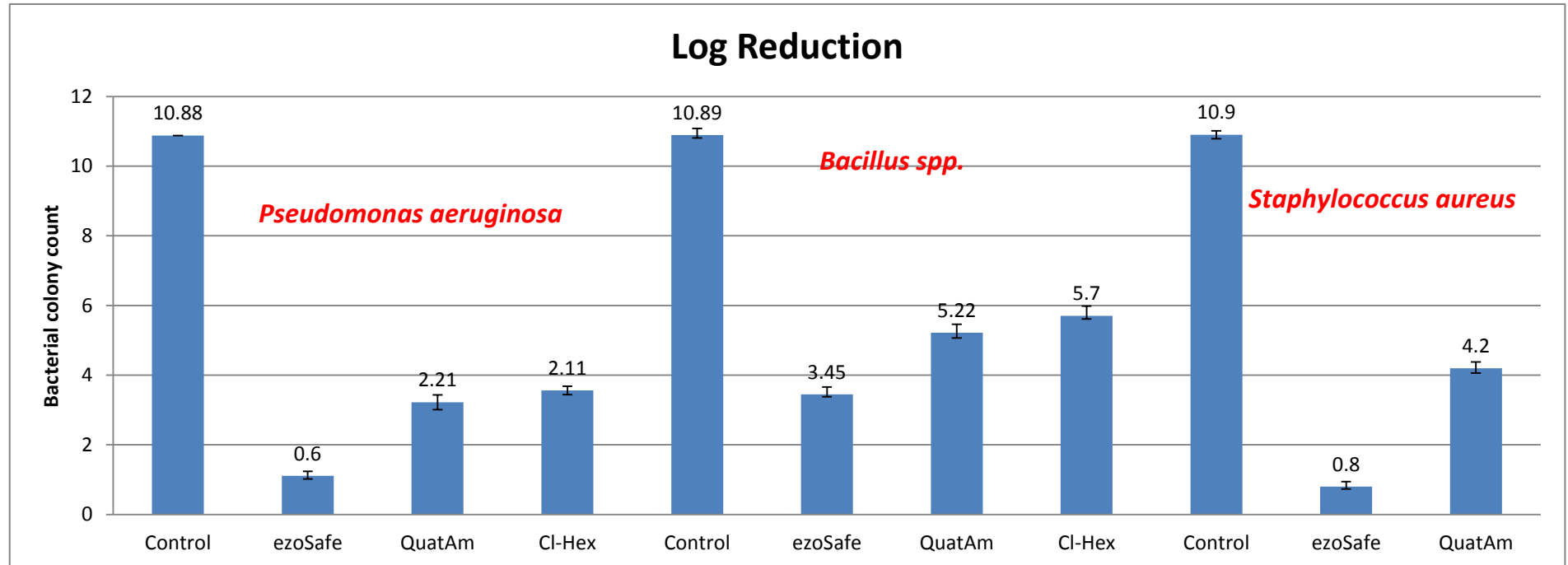
Time Kill Assay:

ezoSafe Time Kill Curve



- Concentration used is MIC x 8 for each product
- Pathogen is *Pseudomonas aeruginosa* at 1.5×10^8 CFU/mL

This is not a reproduction of an actual scenario, as the disinfectant concentrations used are much lower than the manufacturer's specs, however this important assay demonstrates the efficacy of the disinfectant at a very low concentration (8xMIC).

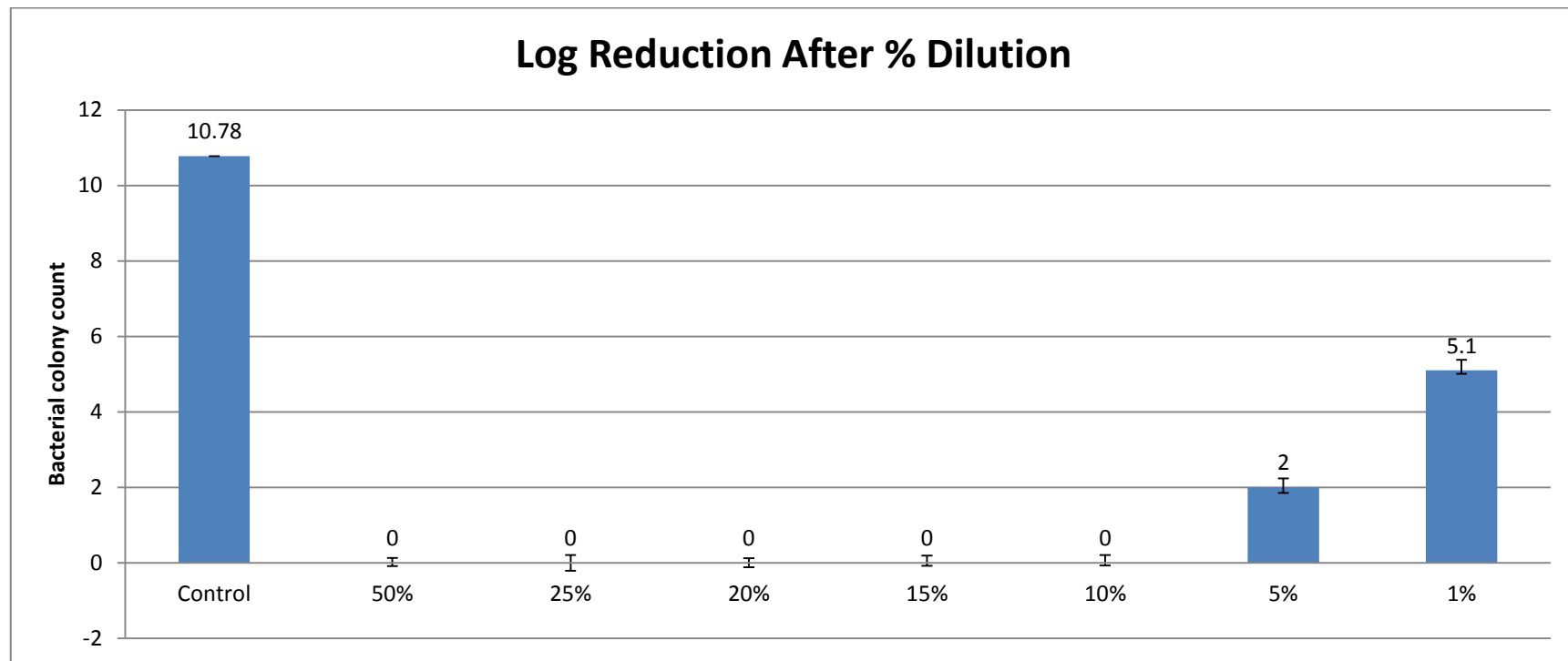


All tests performed at 8 x MIC for each type of disinfectant

Time is 2 hours

This is not a reproduction of an actual scenario, as the disinfectant concentrations used are much lower than the manufacturer's specs, however this important assay demonstrates the efficacy of the disinfectant at a very low concentration (8xMIC).

For the dilution factors, the product was capable to clear a culture of bacterial pathogens at 10 % dilution. As for the 20% dilution, starting from a stock concentration of the disinfectant solution of 5% active ingredient as per manufacturer's specs; corresponds to the concentration of active ingredient used in the Spray bottle of ezoSafe manufacturer (which is 1% active ingredient). This still produces a 10.78 log reduction Even at 5 % dilution, the product remained active with a log reduction of 8.78.



- Above tests are serial dilutions from the 5% active main solution of ezoSafe disinfectant.
- Assay performed as per supplier specs: Spray on surface, wait for 30 seconds then wipe the surface.
- Surface was contaminated with a bacterial colony count of 6×10^{10} Colony Counts, equivalent to $10^{10.78}$.