

ZOONOCIDE APPLICATIONS TO TOUCH POINT SURFACES STOPS THE SPREAD OF BACTERIA

Gram Negative bacteria have been known to cause infection and are responsible for fever-induction. The fever-induction (pyrogenic) effect is due to the presence of an endotoxin within their cell wall. Most Gram Negative bacilli are from human or animal faecal or GI tract origins. Persistent elimination of these harmful bacteria through treatment of various common household articles was demonstrated by treatment with the active Zoonocide material. The following table (TABLE 1) illustrates the reduction in number of gram negative bacteria (reported as Gram Negative Cocci in colony forming units, CFU) from identical treated and untreated surfaces, substrates and articles commonly present in households. Samples were taken by sterile swab, cultured and enumerated. Colony counts were taken on the 1st and 2nd days with the highest counts being taken to calculate final counts. Results are reported in CFU/plate, CFU/area (CFU/plate X Dilution Factor) and CFU/Culture (CFU/area X sample quantity).²⁹

TABLE 1

Comparison of Untreated and Treated Household Items

Article	Untreated Article			Treated Article		
	CFU/plate	CFU/culture	CFU/sq in	CFU/plate	CFU/culture	CFU/sq in
Sink	528	5280	1760	0	0	0
Toilet	53	530	177	0	0	0
Ceramic	548	5480	1830	0	0	0
Countertop						
Shower	432	4320	1440	0	0	0
Curtain						
Stove	393	3930	1310	0	0	0
Wood	432	4320	1440	0	0	0
Cabinet						
Door Knob	393	3930	1310	0	0	0
Stair Rail	25	250	83	0	0	0

To determine efficacy of treatment on different hard, nonporous substrates, granite, garnet, sand, zeolites (silicate mineral), gravel and marble were spray treated with Zoonocide and dried. Chemical testing confirmed all substrates to have a substantial, uniform coating of Zoonocide active material. As all substrates are siliceous materials, zeolites were chosen to be utilized in the studies. Testing was conducted on 3 separate test stations with three challenges per station to determine average microbial reductions.

VIRUSES

Two classes of viruses as represented by bacteriophages MS2 (DNA) and PRD1 (RNA) were selected for this study. Dilute aqueous solutions of the bacteriophages were passed over the treated substrate with a total contact time of <3 minutes/challenge. Three separate challenges (trials) were used to determine an average inactivation for each coliphage. Effluent samples were collected and analyzed. Bacterial hosts *E. coli* and *salmonella typhimurium* were used to detect the MS2 and PRD1 coliphages, respectively.

TABLE F

Viruses Inactivated by Zoonocide Treated Substrate

Average of Challenges as % (log) inactivated

Bacteriophage	LOW	HIGH	Average
MS-2	99.60 (2.40)	99.89 (2.96)	99.84 (2.8)
PRD-1	96.83 (1.50)	99.46 (2.27)	99.00 (2.0)

The difference in inactivation between MS2 and PRD1 can be attributed either to bacteriophage protein coat or to the difference in nucleic acid core (DNA vs. RNA), but not to difference in size (24 nm vs. 63 nm respectively).

BACTERIA

Three independent challenge tests for each of three separate test apparatus for each of the bacteria, *Klebsiella terriena* and *E. coli*, were performed using dilute solutions of the test microbes passed over treated substrates for a total contact time of <3 minutes/challenge. Samples of the water after contact with the treated substrates were collected and examined using membrane filtration (0.45 micron) techniques. Results are reported in the following table.

TABLE G

Bacterial Elimination Using Zoonocide Treated Substrates

Average of Challenges as % (log) Inactivated

Bacteria	LOW	HIGH	Average
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Klebsiella terriena	99.37 (2.2)	99.69 (2.4)	99.50 (2.3)
E. coli	99.96 (3.50)	99.99 (4.39)	99.98 (3.88)

ALGAE/FUNGI

Three separate challenges of three test stations with *Chlorella vulgaris* (ATCC 259220) were conducted. Average contact time of the test solution with the treated substrate for each challenge was <3 minutes. Samples were collected, incubated, enumerated and averaged. Results are reported in the following table.

TABLE H

Fungal Inactivation using Zoonocide Treated Substrates

Average of Challenges as % (log) Inactivated

Algae	LOW	HIGH	Average
<i>Chlorella vulgaris</i>	98.74(1.90)	99.11(2.05)	98.92(1.98)

A separate testing was performed to determine the method of cellular destruction for algae contacting a Zoonocide treated surface. Dilute samples containing *Selenastrum* sp. and *Aphanizomenon* sp. algae were contacted with a treated surface for ~3 minutes. Microscopic examination of the samples collected before contact with the treated surface showed viable algae cells, with their cell walls intact. After contact with the treated substrate, no viable algal cells were identifiable in the collected samples, meaning the cell walls did not appear intact.²²

TABLE I

Lysitic Destruction of Algae

Algae contact	Initial Cell Count	Viable Cell Count after
<i>Selenastrum</i> sp.		
<i>Aphanizomenon</i> sp.	7754/L	0/L

CRYPTOSPORIDIUM PARVUM/PARASITIC PROTOZOA

Three separate challenges of three test units containing Zoonocide treated substrate with infectious *Cryptosporidium parvum* oocysts. This parasitic protozoan is extremely resistant to standard disinfection techniques and chemical agents. It has demonstrated resistance to chlorine disinfection for >24 hours. Average contact time of the test solution with Zoonocide treated substrate for each challenge was <3 minutes. Results of the inactivation of *Cryptosporidium* are reported in Table J.

TABLE J

Inactivation of *Cryptosporidium parvum* Oocysts with Zoonocide Treated Substrate

Pathogen	%Av. Inactivation #1 (log)	%Av. Inactivation #2 (log)	%Av. Inactivation #3 (log)
<i>Cryptosporidium</i> Parvum	95.4(1.34)	99.3(2.15)	98.9(1.96)

The average inactivation for the three test series was 97.9% (1.68 log).

This testing demonstrates a facile, convenient, cost effective method for reducing and inactivating this extremely dangerous pathogen.

The foregoing testing demonstrates the wide range and broad spectrum of Zoonocide treated substrates in inactivating, reducing, eliminating and destroying pathogens without the need for addition of harmful chemical agents into the environment.

The foregoing technology is proprietary to Zono Pty Ltd. Patents Pending.

VIRUS INACTIVATION

Norwalk virus is a single, positive strand RNA, non-enveloped, icosahedral virus of 35-40 nanometre in size. It is a member of the calciviridae family and is similar to Hepatitis E virus and the virus that produces vesicular exanthema of swine.

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While Zoonocide has not been tested to date specifically against the Norwalk virus, it has been extensively tested against the bacteriophage MS2, a structurally similar virus. MS2, like the Norwalk virus, is a positive, single strand RNA, naked type. The bacteriophage MS2 is utilized in testing for safety reasons, as it will only infect bacteria (salmonella) and not vertebrates. Similar to the Norwalk strain, it is one of the simplest virus strains, encoding 4 proteins.

Testing at Arizona State University demonstrated 3 log inactivation of MS2 from water (difficult due to hydration of the virus) with Zoonocide treated substrate. A different type of virus, PRD1, a double strand DNA, non-enveloped virus representative of a rotavirus, was also inactivated in similar numbers. Testing at North Carolina State University using Zoonocide treated media inactivated >3 log (99.9%) MS2 in minutes.

Zoonocide is a broad spectrum antimicrobial that will kill and inactivate a large number of micro-organisms, including viruses, through contact with the pathogen and resultant lyses of the cellular wall.