



Our Experience Is Your Protection

Michelson Laboratories, Inc.

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Maxmyer International, Inc. Challenge Study

August 04, 2011

I. Objective:

To validate the effectiveness of the antimicrobial agent of "Nano Clean" manufactured by Maxmyer International, Inc. against *Salmonella spp* and *E. coli* (including *E. coli* O157:H7) over time at room temperature (72-75F).

II. Procedure:

a. Inoculum Preparation

- i. *Salmonella spp.* (ATCC 14028, 13311, 2524)
- ii. *E. coli*, including *E. coli* O157:H7 (ATCC 25922, 8739, 35150)
- iii. Organisms were purchased from Fisher Scientific and were cultured according to the manufacture's instructions to obtain fresh culture before inoculation. All cultures were grown in Tryptic Soy Broth at 35°C for 24 hours.
- iv. Then the inoculum were used to inoculate D.I water to reach a load of 10^6 - 10^7 cfu/ml.

b. Disinfection treatment

- i. Two dilutions were prepared for Nano Clean solution treatment:
(1) 1g Nano Clean with 100ml of inoculated D.I water (1% concentration),
(2) 1g Nano Clean with 300ml inoculated D.I. Water (0.3% concentration).
The reduction of each pathogen was analyzed on the following time periods:
Control (without treatment), 0 min, 1 hour, 2 hour, 3 hour, 4 hour.

c. Organism analyzes

- i. The sample was plated for *Salmonella spp.* in duplicate using TSA + Y agar overlaid with XLD agar. Incubated at 35°C for 48 hours.
- ii. The sample was plated for *E. coli* in duplicate using TSA + Y agar overlaid with MacConkey with Sorbitol agar. Incubate at 35°C for 24-48 hours.
- iii. Counts were reported as CFU/ml and Log₁₀ CFU/ml.



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III. Reporting

Table1. Survivor counts of *E. coli* (including *E. coli* O157:H7) of **sample diltion1 (1%)** over 4 hours at room temperature (72-75F)

Time (HR)	Survivor level of <i>E. coli</i> (cfu/ml)	Blank (cfu/ml)
Control	38,000,000	38,000,000
0	<10,000	N/A
1	100	N/A
2	<1	N/A
3	<1	N/A
4	<1	38,000,000

Table2. Survivor counts of *Salmonella spp.* of **sample diltion1 (1%)** over 4 hours at room temperature (72-75F)

Time (HR)	Survivor level of <i>Salmonella</i> (cfu/ml)	Blank (cfu/ml)
Control	37,000,000	38,000,000
0	<10,000	N/A
1	19	N/A
2	<1	N/A
3	<1	N/A
4	<1	38,000,000

Table3. Survivor counts of *E. coli* (including *E. coli* O157:H7) of **sample diltion2 (0.3%)** over 4 hours at room temperature (72-75F)

Time (HR)	Survivor level of <i>E. coli</i> (cfu/ml)	Blank (cfu/ml)
Control	16,000,000	14,000,000
0	530,000	N/A
1	2	N/A
2	<1	N/A
3	<1	N/A
4	<1	15,000,000



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Table4. Survivor counts of *Salmonella spp.* of **sample diltion2 (0.3%)** over 4 hours at room temperature (72-75F)

Time (HR)	Survivor level of <i>Salmonella</i> (cfu/ml)	Blank (cfu/ml)
Control	12,000,000	14,000,000
0	720,000	N/A
1	1	N/A
2	<1	N/A
3	<1	N/A
4	<1	15,000,000

IV. Discussion and Conclusion:

It is observed that Nano clean solution has a high effectiveness on reducing counts in *E. coli* and *Salmonella spp.* The efficiency increases with higher concentration. For sample with higher dilution (1%), the result showed an instant decrease for more than 3 log (>99.9%) while dilution (0.3%) only showed 2 log (99%) reduction on time0. After first hour incubation there was no survivor detected from both dilutions. It is concluded that Nano clean with both 1% and 0.3% concentrations can reduce *E. coli* and *Salmonella spp.* for more than 7 log (>99.99999%) within 2 hours of use at room temperature (72-75F).

Microbiology Lab director: Weraugh Date: 8/4/2011