EMSL Analytical, Inc.
Microbiology Division
307 West 38th Street, New York, NY 10018
212-290-0051
www.emsl.com

Certificate of Analysis

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Product: Mesosilver®

Project: Phase II: *Escherichia coli* serotype O157:H7

EMSL Reference: 030401516

Experimental Design Summary:

Test survival of *Escherichia coli* O157:H7 American Type Culture Collection Strain No. 35150 in two Mesosilver products (20 and 75 ppm) using 2 (1 and 10%) concentrations of product as supplied. The organism was tested for survival at 4 (0, 2, 5, and 24 h) time points. A negative control (no product) was included for comparison. All tests were performed in triplicate and plated in duplicate.

Experimental Results Summary:

Escherichia coli O157:H7 at 8.5 x 10^5 cells ml^{-1} was used to determine the effect of Mesosilver on bacterial survival. The results show that Mesosilver has a negative impact on the survival of *E coli* O157:H7 (Tables 1 and 2). Specifically, both concentrations of each product were successful in reducing the numbers of cells to below the level of detection within 24 hours. In addition, the 75 ppm product tested at 10% was able to reduce the numbers to below the level of detection within 5 hours.

Analyst_____ Date: 02-09-04
Lori L. Daane, Ph.D.

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Table 1. Survival of *Escherichia coli* O157:H7 ATCC 35150 inoculated at 8.5 x 10^5 cells ml^{-1} in the presence of 1 and 10% 20 ppm Mesosilver colloidal silver.

20 ppm Mesosilver (%)	CFU ml^{-1}		
	2 h	5 h	24 h
0	$5.1 \times 10^5 \pm 2.6 \times 10^4$	$5.3 \times 10^5 \pm 5.4 \times 10^4$	$4.9 \times 10^5 \pm 5.7 \times 10^3$
1.0	$3.1 \times 10^5 \pm 6.3 \times 10^4$	$7.3 \times 10^3 \pm 1.1 \times 10^3$	<1
10.0	$1.0 \times 10^4 \pm 2.2 \times 10^3$	$2.2 \times 10^2 \pm 8.2 \times 10^1$	<1

All treatments performed in triplicate in 0.35% NaCl incubated without continuous mixing at 35°C. All plate counts performed in duplicate using nutrient agar incubated at 35°C for 72 hours. Results reported as mean ± standard deviation. Media sterility controls showed no growth.

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Table 2. Survival of *Escherichia coli* O157:H7 ATCC 35150 inoculated at 8.5 x 10^5 cells ml^{-1} in the presence of 1 and 10% 75 ppm Mesosilver colloidal silver.

75 ppm Mesosilver (%)	CFU ml^{-1}		
	2 h	5 h	24 h
0	$5.1 \times 10^5 \pm 2.6 \times 10^4$	$5.3 \times 10^5 \pm 5.4 \times 10^4$	$4.9 \times 10^5 \pm 5.7 \times 10^3$
1.0	$4.8 \times 10^4 \pm 8.7 \times 10^2$	$3.0 \times 10^2 \pm 1.0 \times 10^2$	<1
10.0	$4.2 \times 10^3 \pm 5.3 \times 10^2$	<1	<1

All treatments performed in triplicate in 0.35% NaCl incubated without continuous mixing at 35°C. All plate counts performed in duplicate using nutrient agar incubated at 35°C for 72 hours. Results reported as mean ± standard deviation. Media sterility controls showed no growth.

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Raw Data-E coli O157:H7

Treatment	Time Point (hours)					
	2		5		24	
	Colony Count	Dilution Factor	Colony Count	Dilution Factor	Colony Count	Dilution Factor
0.35% Saline-1	60/48	10,000	48/54	10,000	47/50	10,000
0.35% Saline-2	52/47	10,000	56/63	10,000	53/45	10,000
0.35% Saline-3	54/45	10,000	56/43	10,000	45/55	10,000
1.0% 20ppm Mesosilver-1	22/30	10,000	60/95	100	0/0	1
1.0% 20ppm Mesosilver-2	29/28	10,000	60/62	100	0/0	1
1.0% 20ppm Mesosilver-3	39/37	10,000	87/75	100	0/0	1
10% 20 ppm Mesosilver-1	100/110	100	23/17	10	0/0	1
10% 20 ppm Mesosilver-2	125/117	100	41/22	10	0/0	1
10% 20 ppm Mesosilver-3	70/84	100	110/200	1	0/0	1
1.0% 75ppm Mesosilver-1	47/47	1,000	27/10	10	0/0	1
1.0% 75ppm Mesosilver-2	51/46	1,000	40/26	10	0/0	1
1.0% 75ppm Mesosilver-3	54/43	1,000	51/25	10	0/0	1
10% 75ppm Mesosilver-1	37/36	100	0/0	1	0/0	1
10% 75ppm Mesosilver-2	50/44	100	0/0	1	0/0	1
10% 75ppm Mesosilver-3	42/44	100	0/0	1	0/0	1

average colony count x dilution factor = colony forming units per ml