Microbiology Division 338 South Abbott Ave, Milpitas, CA 95035

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www.emsl.com

Certificate of Analysis

Product: Mesosilver®

Project: Antimicrobial effectiveness of colloidal silver

EMSL Reference Number: 090402726

Experimental Design Summary:

Test survival of vancomycin-resistant *Enterococcus faecalis* American Type Culture Collection Strain No. 51299 in two Mesosilver products (20 and 75 ppm) using two (1% and 10%) concentrations of product as supplied. The microorganism was tested for survival at four (0, 2, 5, and 24 hour) time points. A negative control (no product) was included for comparison. The treatments were performed in triplicate in sterile phosphate buffer with MgCl₂ (Hardy Diagnostics) and incubated without continuous mixing at 35°C then plated in duplicate on nutrient agar for 72 hours. Results were reported as mean \pm standard deviation. Media sterility controls showed no growth.

Experimental Results Summary:

Vancomycin-resistant *Enterococcus faecalis* inoculated at 3.6 x 10⁸ cells ml⁻¹ was used to determine the effect of Mesosilver colloidal silver on bacterial survival. The results show that both Mesosilver products have a negative impact on the survival of *E. faecalis* when used at 1% and 10% concentrations (Tables 1 and 2). After 5 hours, 1% and 10% 20 ppm Mesosilver reduced the numbers of *E. faecalis* cells to below the level of detection. Likewise, 1% and 10% 75 ppm Mesosilver reduced the numbers of *E. faecalis* cells to below the level of detection within 5 hours.

| Analyst_ | | Date 10-19-2004 |
|----------|--|-----------------|
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Antimicrobial effectiveness of Mesosilver 20 ppm colloidal silver

| 20 36 3 (24) | CFU ml ⁻¹ | | | |
|-----------------------|---------------------------------------|---------------------------------------|---------------------------------------|--|
| 20 ppm Mesosilver (%) | 2h | 5h | 24h | |
| 0 | $3.7 \times 10^8 \pm 5.8 \times 10^6$ | $3.1 \times 10^8 \pm 1.0 \times 10^6$ | $1.5 \times 10^8 \pm 4.7 \times 10^7$ | |
| 1.0 | $2.5 \times 10^8 \pm 2.5 \times 10^7$ | <1 | <1 | |
| 10.0 | $1.9 \times 10^8 \pm 3.5 \times 10^7$ | <1 | <1 | |

Table 1. Survival of vancomycin-resistant *Enterococcus faecalis* American Type Culture Collection Strain No. 51299 inoculated at 3.6 x 10⁸ cells ml⁻¹ in the presence of 1% and 10% 20 ppm Mesosilver colloidal silver.

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Antimicrobial effectiveness of Mesosilver 75 ppm colloidal silver

| 75 M 1 (0/) | CFU ml ⁻¹ | | | | |
|-----------------------|---------------------------------------|---------------------------------------|---------------------------------------|--|--|
| 75 ppm Mesosilver (%) | 2h | 5h | 24h | | |
| 0 | $3.7 \times 10^8 \pm 5.8 \times 10^6$ | $3.1 \times 10^8 \pm 1.0 \times 10^6$ | $1.5 \times 10^8 \pm 4.7 \times 10^7$ | | |
| 1.0 | $1.4 \times 10^8 \pm 1.2 \times 10^7$ | <1 | <1 | | |
| 10.0 | $1.3x\ 10^8 \pm 7.9x\ 10^6$ | <1 | <1 | | |

Table 2. Survival of vancomycin-resistant *Enterococcus faecalis* American Type Culture Collection Strain No. 51299 inoculated at 3.6 x 10⁸ cells ml⁻¹ in the presence of 1% and 10% 75 ppm Mesosilver colloidal silver.

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Antimicrobial effectiveness of Mesosilver colloidal silver

| | Time Point (hours) | | | | | |
|-------------------------------|--------------------|----------|--------|----------|--------|----------|
| Treatment | 2 | | 5 | | 24 | |
| Treatment | Colony | Dilution | Colony | Dilution | Colony | Dilution |
| | Count | Factor | Count | Factor | Count | Factor |
| 1.0% 20 ppm Mesosilver-1 | 258 / 241 | 1000000 | 0/0 | 1000000 | 0/0 | 1000000 |
| 1.0% 20 ppm Mesosilver –2 | 281 / 282 | 1000000 | 0/0 | 1000000 | 0/0 | 1000000 |
| 1.0% 20 ppm Mesosilver -3 | 240 / 224 | 1000000 | 0/0 | 1000000 | 0/0 | 1000000 |
| 10% 20 ppm Mesosilver –1 | 134 / 204 | 1000000 | 0/0 | 1000000 | 0/0 | 1000000 |
| 10% 20 ppm Mesosilver -2 | 235 / 230 | 1000000 | 0/0 | 1000000 | 0/0 | 1000000 |
| 10% 20 ppm Mesosilver -3 | 184 / 169 | 1000000 | 0/0 | 1000000 | 0/0 | 1000000 |
| 1.0% 75 ppm Mesosilver –1 | 147 / 148 | 1000000 | 0/0 | 1000000 | 0/0 | 1000000 |
| 1.0% 75 ppm Mesosilver -2 | 147 / 155 | 1000000 | 0/0 | 1000000 | 0/0 | 1000000 |
| 1.0% 75 ppm Mesosilver –3 | 127 / 131 | 1000000 | 0/0 | 1000000 | 0/0 | 1000000 |
| 10.0% 75 ppm Mesosilver –1 | 126 / 132 | 1000000 | 0/0 | 1000000 | 0/0 | 1000000 |
| 10% 75 ppm Mesosilver –2 | 133 / 145 | 1000000 | 0/0 | 1000000 | 0/0 | 1000000 |
| 10% 75 ppm Mesosilver -3 | 120 / 127 | 1000000 | 0/0 | 1000000 | 0/0 | 1000000 |

Table 3. Raw data of antimicrobial effectiveness of colloidal silver against vanomycin resistant *Enterococcus faecalis*. Average colony count x dilution factor = colony forming units per ml (CFU ml⁻¹).