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**Zetrisol**<sup>TM</sup>

A PROPRIETARY SILICONE QUATERNARY SOLUTION

## **“ZETRISIL” NANO SILICONE-BASED HAND SANITIZERS ARE PROVEN TO REDUCE MICROBIAL LOADING ON THE HANDS FOR EXTENDED PERIODS, THUS REDUCING THE THREAT OF HBI (HOSPITAL BORN INFECTION)**

My-shield<sup>®</sup> Hand Santizer recently participated in an extensive third-party sponsored 26 staff member study at a prominent US-based hospital. The goal was to test the potential effectiveness and persistence of the product(s) and record any reduction of bacteria loadings on the hands as compared to current alcohol sanitizing and cleaning protocols currently adopted by the medical facility.

**Sponsor:** Highmark Inc. “Medical Division” 501 Penn Ave, Pittsburgh, PA 15222

Enviro Specialty Chemicals, Inc. 4999 43 St SE, Calgary, Suite #171, AB T2B 3N4

**Applicator:** Pelican Solutions Group, Wexford, PA 15090

**Medical facility:** Wheeling Mission Hospital, 1 Medical Park, Wheeling West Virginia 26003

**Microbial testing laboratory:** Wheeling Mission Hospital, “Analytical Department”  
1 Medical Park, Wheeling West Virginia 26003

Eurofins Microbiology Laboratories, Inc.  
2430 New Holland Pike Lancaster, PA 17601

**Objectives of the study:** To quantify the effectiveness of the My-shield<sup>®</sup> nano silicone hand sanitizer technology in reducing microbial loadings on the hands in a working hospital environment over current hand sanitization protocols & the extent of microbial reduction persistence under laboratory conditions on porcine skin.

**Study data summary:** The working hospitals study was conducted over 2 days on 26 hospital staff members with 104 microbial labs testing & the laboratory testing was conducted under GLP conditions for 4 & 24 hrs against known ATCC registered strains of bacteria.

## 4 HR STUDY AGAINST ANTIBIOTIC RESISTANT MICROORGANISMS

Methicillin-resistant *Staphylococcus aureus* (MRSA; ATCC #33592) Vancomycin-resistant *Enterococcus faecalis* (VRE; ATCC #51575) Carbapenem-resistant *Enterobacteriaceae* (CRE; *Klebsiella pneumonia* ATCC #BAA-1705)

### Data analysis

The raw count observed for each treated sample was compared to the untreated control. The percent reduction between the treated and untreated samples was defined as the efficacy of each treatment at each time point.

### Challenge organism concentrations

Results of the enumeration of the challenge organisms are shown in Table 1 below.

Table 1. Challenge organism enumeration

| Challenge organism | Sample 1    | Sample 2    | Sample 3    | Average     | Log <sub>10</sub> |
|--------------------|-------------|-------------|-------------|-------------|-------------------|
| MRSA (cfu/mL)      | 218,000,000 | 153,000,000 | 147,000,000 | 172,666,667 | 8.24              |
| VRE (cfu/mL)       | 144,000,000 | 175,000,000 | 81,000,000  | 133,333,333 | 8.12              |
| CRE (cfu/mL)       | 195,000,000 | 77,000,000  | 58,000,000  | 110,000,000 | 8.04              |

The overnight culture suspensions were at a concentration of 1.72 x 10<sup>8</sup> cfu/mL for MRSA, 1.33 x 10<sup>8</sup> cfu/mL for VRE, and 1.10 x 10<sup>8</sup> cfu/mL for CRE. All cultures were used undiluted as inocula in the study.

### Porcine skin sample results

Results of the challenge samples are shown in Tables 2 through 4, below, including holding time, type of sample, sample replicate, amount of inoculum recovered (in cfu/mL of rinsate), and the percent reduction seen

Table 2. Porcine skin sample enumeration (MRSA)

| Holding Time (Sample Type) | Sample 1    | Sample 2   | Sample 3   | Average    | Log <sub>10</sub> | % Reduction |
|----------------------------|-------------|------------|------------|------------|-------------------|-------------|
| 2 minutes (Untreated)      | 104,000,000 | 45,000,000 | 22,500,000 | 57,166,667 | 7.76              | 99.9%       |
| 2 minutes (Treated)        | 52,000      | 65,000     | 56,000     | 57,667     | 4.76              |             |
| 1 hour (Untreated)         | 14,400,000  | 3,600,000  | 6,400,000  | 8,133,333  | 6.91              | 99.2%       |
| 1 hour (Treated)           | 46,000      | 79,000     | 61,000     | 62,000     | 4.79              |             |
| 2 hours (Untreated)        | 19,800,000  | 21,900,000 | 12,200,000 | 17,966,667 | 7.25              | 97.4%       |
| 2 hours (Treated)          | 600,000     | 610,000    | 208,000    | 472,667    | 5.67              |             |
| 4 hours (Untreated)        | 17,600,000  | 14,700,000 | 6,300,000  | 12,866,667 | 7.11              | 95.2%       |
| 4 hours (Treated)          | 850,000     | 360,000    | 650,000    | 620,000    | 5.79              |             |
| 24 hours (Uninoculated)    | <1          | <1         | <1         | <1         | n/a               | n/a         |

Table 3. Porcine skin sample enumeration (VRE)

| Holding Time (Sample Type) | Sample 1   | Sample 2   | Sample 3   | Average    | Log <sub>10</sub> | % Reduction |
|----------------------------|------------|------------|------------|------------|-------------------|-------------|
| 2 minutes (Untreated)      | 10,600,000 | 63,000,000 | 71,000,000 | 48,200,000 | 7.68              | 99.9%       |
| 2 minutes (Treated)        | 90,000     | 19,800     | 16,200     | 42,000     | 4.62              |             |
| 1 hour (Untreated)         | 10,300,000 | 20,500,000 | 11,600,000 | 14,133,333 | 7.15              | 99.4%       |
| 1 hour (Treated)           | 157,000    | 28,000     | 66,000     | 83,667     | 4.92              |             |
| 2 hours (Untreated)        | 18,500,000 | 6,300,000  | 6,500,000  | 10,433,333 | 7.02              | 98.1%       |
| 2 hours (Treated)          | 162,000    | 107,000    | 330,000    | 199,667    | 5.30              |             |
| 4 hours (Untreated)        | 11,300,000 | 3,400,000  | 21,200,000 | 11,966,667 | 7.08              | 90.9%       |
| 4 hours (Treated)          | 1,690,000  | 970,000    | 620,000    | 1,093,333  | 6.04              |             |
| 24 hours (Uninoculated)    | <1         | <1         | <1         | <1         | n/a               | n/a         |

Table 4. Porcine skin sample enumeration (CRE)

| Holding Time (Sample Type)                   | Sample 1                | Sample 2               | Sample 3                | Average                 | Log <sub>10</sub> | % Reduction |
|--|-------------------------|------------------------|-------------------------|-------------------------|-------------------|-------------|
| 2 minutes (Untreated)<br>2 minutes (Treated) | 15,500,000<br>126,000   | 65,000,000<br>122,000  | 18,100,000<br>145,000   | 32,866,667<br>131,000   | 7.52<br>5.12      | 99.6%       |
| 1 hour (Untreated)<br>1 hour (Treated)       | 19,100,000<br>169,000   | 6,400,000<br>149,000   | 2,800,000<br>177,000    | 9,433,333<br>165,000    | 6.97<br>5.22      | 98.3%       |
| 2 hours (Untreated)<br>2 hours (Treated)     | 7,400,000<br>1,110,000  | 17,300,000<br>970,000  | 7,400,000<br>176,000    | 10,700,000<br>752,000   | 7.03<br>5.88      | 93.0%       |
| 4 hours (Untreated)<br>4 hours (Treated)     | 20,900,000<br>2,120,000 | 8,300,000<br>2,160,000 | 16,100,000<br>1,190,000 | 15,100,000<br>1,823,333 | 7.18<br>6.26      | 87.9%       |
| 24 hours (Uninoculated)                      | <1                      | <1                     | <1                      | <1                      | n/a               | n/a         |

Efficacy of the treatment dropped from a maximum of 99.9 – 99.6% after 2 minutes of storage to a minimum of 95.2 – 87.9% after 4 hours of the study. The treatment was most effective against MRSA, with efficacy ranges from 99.9% to 95.2%, while the treatment was least effective against CRE, with efficacy ranges from 99.6% to 87.9% over the course of the study. Representative isolates were confirmed as their respective inocula. No background organisms were detected in the uninoculated samples over the course of the study.



## 24 HR STUDY AGAINST STAPHYLOCOCCUS AUREUS (ATCC #12600)

Results of the challenge samples are shown in Table 1, below, including holding time, type of sample, sample replicate, amount of *S. aureus* recovered (in cfu/mL of rinsate), and the percent reduction seen between the treated and untreated samples at each time point.

| Holding Time (Sample Type)                   | Sample 1                | Sample 2                | Average                 | Log <sub>10</sub> | % Reduction |
|--|-------------------------|-------------------------|-------------------------|-------------------|-------------|
| 2 minutes (Untreated)<br>2 minutes (Treated) | 22,400,000<br>11,200    | 16,400,000<br>13,900    | 19,400,000<br>12,550    | 7.29<br>4.10      | 99.9%       |
| 1 hour (Untreated)<br>1 hour (Treated)       | 5,200,000<br>87,000     | 18,800,000<br>164,000   | 12,000,000<br>125,500   | 7.08<br>5.10      | 99.0%       |
| 2 hours (Untreated)<br>2 hours (Treated)     | 13,900,000<br>137,000   | 15,900,000<br>380,000   | 14,900,000<br>258,500   | 7.17<br>5.41      | 98.3%       |
| 4 hours (Untreated)<br>4 hours (Treated)     | 8,200,000<br>156,000    | 17,200,000<br>640,000   | 12,700,000<br>398,000   | 7.10<br>5.60      | 96.9%       |
| 8 hours (Untreated)<br>8 hours (Treated)     | 9,500,000<br>1,580,000  | 13,700,000<br>1,930,000 | 11,600,000<br>1,755,000 | 7.06<br>6.24      | 84.9%       |
| 16 hours (Untreated)<br>16 hours (Treated)   | 3,700,000<br>1,770,000  | 14,900,000<br>2,040,000 | 9,300,000<br>1,905,000  | 6.97<br>6.28      | 79.5%       |
| 24 hours (Untreated)<br>24 hours (Treated)   | 10,500,000<br>3,400,000 | 15,400,000<br>8,500,000 | 12,950,000<br>5,950,000 | 7.11<br>6.77      | 54.1%       |
| 24 hours (Uninoculated)                      | <1                      | <1                      | <1                      | n/a               | n/a         |

Efficacy of the treatment dropped from a maximum of 99.9% to a minimum of 54.1% of the 24 hours of the study. Representative isolates were confirmed as the inoculum (*S. aureus*). No background *S. aureus* was detected in the uninoculated samples over the course of the study.

# WHEELING MISSION HEALTH CARE WORKERS 2 DAY STUDY RESULTS

## Hospital Staff Members Group 1

| Subject | Baseline  | Follow-up   | Follow-up  | Follow-up   |
|---------|---|---|--|---|
| 1       | 2 Colonies (CFUs) Micrococcus Species; 6 Colonies (CFUs) Coagulase Negative Staphylococcus; 18 Colonies (CFUs) Coagulase Negative Staphylococcus NUMBER 2 | 5 Colonies (CFUs) Coagulase Negative Staphylococcus   | No Growth Day 2  | 15 Colonies (CFUs) Coagulase Negative Staphylococcus; 8 Micrococcus Species |
| 5       | 2 Colonies (CFUs) Coagulase Negative Staphylococcus; 1 Colonies (CFUs) Micrococcus Species  | 5 Colonies (CFUs) Coagulase Negative Staphylococcus   | 3 Colonies (CFUs) Coagulase Negative Staphylococcus  | 26 Colonies (CFUs) Coagulase Negative Staphylococcus                        |
| 9       | 6 Colonies (CFUs) Coagulase Negative Staphylococcus   | 1 Colonies (CFUs) Coagulase Negative Staphylococcus   | No Growth Day 2  | 2 Colonies (CFUs) Coagulase Negative Staphylococcus                         |
| 13      | 4 Colonies (CFUs) Coagulase Negative Staphylococcus   | 2 Colonies (CFUs) Coagulase Negative Staphylococcus; 5 Colonies (CFUs) Coagulase Negative Staphylococcus NUMBER 2; 1 Colonies (CFUs) Coagulase Negative Staphylococcus NUMBER 3 | 2 Colonies (CFUs) Coagulase Negative Staphylococcus; 2 Colonies (CFUs) Filamentous Fungus  | n/a   |
| 17      | 7 Colonies (CFUs) Coagulase Negative Staphylococcus   | No Growth Day 2   | No Growth Day 2  | No Growth Day 2   |
| 23      | No Growth Day 2   | 3 Colonies (CFUs) Coagulase Negative Staphylococcus; 3 Colonies (CFUs) Coagulase Negative Staphylococcus NUMBER 2; 2 Colonies (CFUs) Coagulase Negative Staphylococcus NUMBER 3 | No Growth Day 2  | No Growth Day 2   |
| 25      | No Growth Day 2   | 1 Colonies (CFUs) Diphtheroids; 2 Colonies (CFUs) Coagulase Negative Staphylococcus   | 125 Colonies (CFUs) Streptococci, Alpha Hemolytic; 12 Colonies (CFUs) Coagulase Negative Staphylococcus; 1 Colonies (CFUs) Micrococcus Species | n/a   |

## Hospital Staff Members Group 2

| Subject | Baseline  | Follow-up   | Follow-up   | Follow-up   |
|---------|---|---|---|---|
| 2       | 2 Colonies (CFUs) Staphylococcus Aureus; 3 Colonies (CFUs) Coagulase Negative Staphylococcus NUMBER 2; 8 Colonies (CFUs) Coagulase Negative Staphylococcus NUMBER 3 | No Growth Day 2                                     | 200 Colonies (CFUs) Diphtheroids                    | 1 Colonies (CFUs) Micrococcus Species               |
| 6       | No Growth Day 2   | 1 Colonies (CFUs) Coagulase Negative Staphylococcus | No Growth Day 2                                     | n/a   |
| 10      | No Growth Day 2   | No Growth Day 2                                     | No Growth Day 2                                     | n/a   |
| 15      | 8 Colonies (CFUs) Micrococcus Species; 8 Colonies (CFUs) Coagulase Negative Staphylococcus; 2 Diphtheroids  | No Growth Day 2                                     | No Growth Day 2                                     | 1 Colonies (CFUs) Coagulase Negative Staphylococcus |
| 18      | 3 Colonies (CFUs) Coagulase Negative Staphylococcus; 2 Colonies (CFUs) Micrococcus Species  | No Growth Day 2                                     | 1 Colonies (CFUs) Coagulase Negative Staphylococcus | No Growth Day 2                                     |
| 22      | No Growth Day 2   | 5 Colonies (CFUs) Coagulase Negative Staphylococcus | 3 Colonies (CFUs) Micrococcus Species               | 2 Colonies (CFUs) Coagulase Negative Staphylococcus |
| 26      | 1 Colonies (CFUs) Coagulase Negative Staphylococcus   | 2 Colonies (CFUs) Coagulase Negative Staphylococcus | No Growth Day 2                                     | No Growth Day 2                                     |

## Hospital Staff Members Group 3

| Subject | Baseline   | Follow-up  | Follow-up   | Follow-up  |
|---------|--|--|---|--|
| 4       | No Growth Day 2  | 1 Colonies (CFUs) Coagulase Negative Staphylococcus  | 3 Colonies (CFUs) Coagulase Negative Staphylococcus | n/a  |
| 7       | 19 Colonies (CFUs) Coagulase Negative Staphylococcus; 1 Colonies (CFUs) Micrococcus Species  | 14 Colonies (CFUs) Coagulase Negative Staphylococcus   | 1 Colonies (CFUs) Diphtheroids                      | n/a  |
| 11      | 2 Colonies (CFUs) Micrococcus Species  | 1 Colonies (CFUs) Coagulase Negative Staphylococcus; 7 Colonies (CFUs) Coagulase Negative Staphylococcus NUMBER 2; 14 Colonies (CFUs) Coagulase Negative Staphylococcus NUMBER 3 | No Growth Day 2                                     | n/a  |
| 14      | 3 Colonies (CFUs) Coagulase Negative Staphylococcus  | 1 Colonies (CFUs) Coagulase Negative Staphylococcus; 5 Colonies (CFUs) Coagulase Negative Staphylococcus   | n/a   | n/a  |
| 19      | 2 Colonies (CFUs) Coagulase Negative Staphylococcus; 1 Colonies (CFUs) Coagulase Negative Staphylococcus NUMBER 1  | 2 Colonies (CFUs) Micrococcus Species ; 1 Colonies (CFUs) Coagulase Negative Staphylococcus  | No Growth Day 2                                     | 11 Colonies (CFUs) Coagulase Negative Staphylococcus |
| 24      | 2 Colonies (CFUs) Coagulase Negative Staphylococcus; 2 Colonies (CFUs) Streptococci, Alpha Hemolytic; 1 Colonies (CFUs) Coagulase Negative Staphylococcus NUMBER 2 | 1 Colonies (CFUs) Coagulase Negative Staphylococcus; Gram Positive Cocci Colonies (CFUs) Micrococcus Species   | 1 Colonies (CFUs) Coagulase Negative Staphylococcus | No Growth Day 2                                      |

## Hospital Staff Members Group 4

| Subject | Baseline  | Follow-up   | Follow-up   | Follow-up   |
|---------|---|---|---|---|
| 3       | 3 Colonies (CFUs) Coagulase Negative Staphylococcus; 1 Colonies (CFUs) Coagulase Negative Staphylococcus NUMBER 2; 5 Colonies (CFUs) Coagulase Negative Staphylococcus NUMBER 2; 21 Colonies (CFUs) Micrococcus Species | 2 Colonies (CFUs) Coagulase Negative Staphylococcus; 3 Colonies (CFUs) Micrococcus species  | n/a   | n/a   |
| 8       | 1 Colonies (CFUs) Bacillus Species; 1 Colonies (CFUs) Coagulase Negative Staphylococcus; 1 Filamentous Fungus   | No Growth Day 2   | No Growth Day 2                                     | 4 Colonies (CFUs) Coagulase Negative Staphylococcus |
| 12      | 2 Colonies (CFUs) Coagulase Negative Staphylococcus; 1 Colonies (CFUs) Coagulase Negative Staphylococcus NUMBER 2   | 1 Colonies (CFUs) Coagulase Negative Staphylococcus   | No Growth Day 2                                     | n/a   |
| 16      | 2 Colonies (CFUs) Coagulase Negative Staphylococcus; 1 Colonies (CFUs) Coagulase Negative Staphylococcus NUMBER 2; 1 Colonies (CFUs) Coagulase Negative Staphylococcus NUMBER 3   | 4 Colonies (CFUs) Coagulase Negative Staphylococcus; 2 Colonies (CFUs) Coagulase Negative Staphylococcus NUMBER 2; 1 Colonies (CFUs) Coagulase Negative Staphylococcus NUMBER 3 | 3 Colonies (CFUs) Coagulase Negative Staphylococcus | n/a   |
| 20      | 2 Colonies (CFUs) Micrococcus Species; 1 Colonies (CFUs) Coagulase Negative Staphylococcus  | 4 Colonies (CFUs) Micrococcus Species   | No Growth Day 2                                     | n/a   |
| 21      | 1 Colonies (CFUs) Diphtheroids  | 2 Colonies (CFUs) Streptococci, Alpha Hemolytic; 13 Colonies (CFUs) Coagulase Negative Staphylococcus; 8 Colonies (CFUs) Coagulase Negative Staphylococcus NUMBER 2             | No Growth Day 2                                     | n/a   |

## CONCLUSIONS:

### **Eurofins 4hr study against MRSA (ATCC #33592), VRE ATCC #51575) & CRE (ATCC #BAA-1705) on porcine skin**

The data in this study indicates that My-shield® Hand Sanitizer was able to reduce the growth of the test organisms (Methicillin-resistant Staphylococcus aureus (MRSA) ATCC #33592, Vancomycin-resistant Enterococcus faecalis (VRE) ATCC #51575, and Carbapenem-resistant Enterobacteriaceae (CRE); Klebsiella pneumonia ATCC #BAA-1705) for extended time periods, especially against MRSA and VRE. The calculated percent efficacy of the treatment peaked at 99.9% (i.e. 3 logs) and decreased to an average of 91.3% reduction at 4hrs

### **Eurofins 24 hr study against Staphylococcus aureus (ATCC #12600) on porcine skin**

The data in this study indicates that the My-shield® Hand Sanitizer was able to reduce the growth of the test organism (Staphylococcus aureus ATCC #12600) for extended time periods. The calculated percent efficacy of the treatment peaked at 99.9% (i.e. 3 logs of reduction) 2 minutes after application. Percent efficacy remained above 99.0% (2 logs of reduction) for up to an hour after application, and remained above 90.0% (1 log of reduction) for up to 4 hours after application. Percent efficacy decreased gradually to 54% at 24hrs.

### **Wheeling Mission Health Care Workers 2 day study**

In a Hand Study Test of 26 Health Care Workers conducted by the Infectious Disease Department with one application only of My-shield® Hand sanitizer, the results were favorable in reducing total bacterial loadings on the hands.

Hands were swabbed for a baseline microorganism loading in the morning between 7:00 and 9:15 am and cultured. Of the 26 Health Care Workers, 5 of the 26 showed no growth on their hands; while 21 showed positive for growths of CFUs. The typical microorganisms were listed as Coagulase Negative Staphylococcus (CNSs), Micrococcus species, Bacillus species, Filamentous Fungi, Streptococci Alpha Hemolytic, and Diphtheroids. Various loadings of CFUs were noted. My-shield® Hand Sanitizer was applied once and allowed to dry. The test was to observe the persistence of one application of hand sanitizer over a 8 hour period.

A second swabbing of the hands occurred from 9:50 to 10:50 am. During this observation 6 Health Care Workers had no growth.

A third swabbing of the hands occurred from 12:40 to 1:55 pm. During this observation 14 of the 26 Health Care Workers exhibited no growth on the plates.

A fourth swabbing of the hands occurred from 1:50 to 3:45 pm. During this observation 5 of the remaining 13 Health Care Workers exhibited no growth on the plates while 13 of the 26 Health Care Workers did not participate due to shift changes.

During this test there were 6 occurrences of higher than expected loadings on the hands of health care workers such as 200 CFU of Diphtheroids, 26 CFU of CNSs and 1 CFU of Filamentous Fungi.

The test showed that transient bacteria can contaminate hands during a 8 hour shift with only one application of My-shield® hand sanitizer (allow no growth was evident on occasions) and standard protocols of washing the hands followed by application of my-shield hand sanitizer after each patient contact would no doubt reduce overall bacterial loading on the hands and reduce the potential for HBI.