

Dri-Mist™ Treatments in Fire Rescue Unit #16 and on a Fireman's Jacket

An Assessment of the Effectiveness of Zimek's Room and Vehicle Sterilizing System in Killing Bacteria, Including Dangerous Methicillin Resistant Staphylococcus Aureus Bacteria ("MRSA")

Initiation of Project: September 5, 2007

Report Date: October 5, 2007

OVERVIEW – MRSA FOUND IN FIRE RESCUE UNIT #16 AND ON FIREMAN'S JACKET

This study was undertaken to examine the effectiveness of a room and vehicle sterilizing system ("System") manufactured by Zimek Technologies, LLC ("Zimek") to kill high concentrations of bacteria, including the potentially deadly MRSA, which were found in Fire Rescue Unit #16 of the ██████████ County, Florida Fire Department (and on a fireman's jacket contained therein). The Zimek System produces negatively charged micro-particles of Zimek's microbicidal QD disinfectant called "Dri-Mist,™" which was vented into the rear window of Fire Rescue Unit #16 sterilizing the interior air and disinfecting surface areas wherever free air flowed in the rescue unit, including on a fireman's jacket located in the vehicle. The specific goal was to determine the bactericidal effectiveness of the quaternary ammonium "high efficiency" Zimek Dri-Mist™ treatment under conditions of high bacterial levels thereby demonstrating that continued use of the Zimek System provides a healthier environment for firefighters, paramedics, and patients who occupy fire rescue units. Swab tests were performed in Fire Rescue Unit #16, and on the fireman's jacket contained therein, at ██████████ County's Fire Station #16 located in ██████████, Florida. The initial swab test was performed on September 5, 2007 and a follow-up study (final swab test) was performed ten days later on September 15, 2007.

SIGNIFICANCE – MRSA INFECTIONS CAN BE DEADLY

MRSA infections are an impending epidemic that threaten the health and safety of all firefighters, paramedics, and their patients. MRSA stands for *methicillin resistant staphylococcus aureus*, a dangerous bacteria that can cause drug resistant staph infections. Until the late 1990s, MRSA was found exclusively in hospitals. Beginning in 1998, a community-associated form of the bacteria (CA-MRSA) emerged globally, with more potential toxins than hospital-acquired MRSA.¹

Warnings about MRSA have become more prevalent during recent years as the bacteria has become more resistant to antibiotics. MRSA has become more difficult to treat and frequently leads to death. The infections caused by MRSA used to be contained only in hospitals and nursing homes, where many patients are sick or elderly. Lately, MRSA has started to infect even the strongest individuals, such as firefighters.²

A recent research study published in the Pre-Hospital Emergency Care Journal examined the presence of MRSA in an ambulance fleet of 21 vehicles. MRSA is a strain of bacteria that is resistant to beta-lactam antibiotics such as methicillin, amoxicillin, and penicillin, which are commonly used to treat bacterial infections. The research found MRSA contamination in 10 of 21 ambulances. Some of the areas that tested positive for MRSA growth were the steering wheel, left patient stretcher handrail, patient stretcher cushion, work area to the right of the patient, and the

¹ "An impending epidemic: MRSA," Prerna Grover, May 29, 2007, http://www.themoneytimes.com/articles/20070529/an_impending_epidemic_mrsa-id-104265.html

² "Bacterial scare: Tucson Fire taking extra care after four infected," Heidi Rowley, Tuesday, December 20, 2005, www.tusconcitizen.com

Yankauer suction tip. The authors of the study concluded that the ambulance environment may be significantly contaminated with MRSA and that the EMS system could represent an important reservoir in the transmission of MRSA to patients. Firefighters and paramedics are at risk of being infected by MRSA in the line of duty and becoming MRSA carriers unknowingly transporting MRSA home to their families.^{3&4} The instant study confirmed the existence of MRSA in Fire Rescue Unit #16 and on the fireman's jacket located therein.

METHODOLOGY OF TESTING AND DRI-MIST™ TREATMENTS

1. September 5, 2007 – Initial Testing of Fire Rescue Unit #16, including Fireman's Jacket

The following areas of Fire Rescue Unit #16 were marked with a numbered one inch square piece of blue masking tape: (27) Steering wheel; (28) Floor [driver's side]; (29) Driver's door handle area; (30) Driver's seat; (31) Air conditioning return duct; (32) Right side door handle; (33) Needle drawer opening; (34) Red EMT seat; (35) Gear bag strap; (36) Gurney lever; (37) Ceiling by light; and (38) Fireman's jacket.

A. Before Dri-Mist™ Treatment – 1st Swab Test

Fire Rescue Unit #16 had been cleaned manually daily between 7 and 8 AM by the fire rescue crew on duty. A microbiologist from Microbac Laboratories, Inc., ("Microbac") used sterile sponges containing 10 mL of Lethen Broth with sterile four inch by four inch (4" x 4") templates to obtain bacterial samples from each area.⁵ A sponge sample of sixteen square inches was taken to the left of the numbered tape thirty minutes prior to Zimek's Dri-Mist™ treatment. The pre-treatment sponge samples were taken by the microbiologist at 10:30 AM.

Examples of Treatment Areas being Swabbed:

27. Steering Wheel



28. Driver's Seat



33. Needle Drawer Opening



38. Fireman's Jacket



³ "MRSA Colonization in Ambulances," RapidCE June, 2007 Newsletter

⁴ "MRSA in the Ambulance," Kristi L. Koenig, MD, FACEP, Published in Journal Watch Emergency Medicine, June 8, 2007

⁵ The sterile sponges, manufactured by Biotrace International, were obtained from 3M Company. Each contained 10 mL of Lethen Broth. ID#VEN0019-432; Lot #H507223; Expiration date: 7/10/2008; ISO 9001-2000 Certified

B. During and After Dri-Mist™ Treatment – 2nd Swab Test

Zimek's System was used at 11:00 AM for thirty minutes venting Zimek's Dri-Mist™ treatment through the window of the rear entry door of the vehicle while the front and rear air conditioning systems were not operating.

The 30 minute Dri-Mist™ treatment was followed by a ten minute "dwell time" to maximize the application of the Dri-Mist™ disinfecting micro-particles to all interior surface areas. The front and rear doors were then opened to quickly vent the residual airborne Dri-Mist™ micro-particles from Fire Rescue Unit #16 for five minutes.

The Dri-Mist™ treatment is a dry disinfectant application which allowed the microbiologist from Microbac to immediately take a second sponge sample of sixteen square inches taken to the right of each piece of numbered tape (post-treatment sponge samples).

The twelve pre-treatment and twelve post-treatment samples were placed in a cooler and immediately transported to Microbac's laboratory in Venice, Florida. The temperature of the swabs upon return to the laboratory was 10° C. Analysis of the swab samples began seven hours after the samples were taken. The laboratory analysis compared the levels of pre-existing bacterial contaminants to the levels after the Zimek Dri-Mist™ treatment. Each swab was analyzed for:

1. Heterotrophic plate count (total bacterial level);
2. Staphylococcus aureus bacteria count;
3. MRSA bacteria (methicillin-resistant staphylococcus aureus) count, if staphylococcus was present at site.

2. September 6-8 and 12, 2007 – Intermediate Dri-Mist™ Treatments

A fire rescue unit crew performed additional Dri-Mist™ treatments after the initial September 5, 2007 treatment on:

- September 6, 2007 = 10 minutes;
- September 7, 2007 = 10 minutes;
- September 8, 2007 = 12 minutes;
- September 12, 2007 = 10 minutes.⁶

3. September 15, 2007 – 3rd Swab test (No Dri-Mist™ Treatments)

Ten days later on September 15, 2007, sponge samples were taken in the same treatment areas of Fire Rescue Unit #16 by the same microbiologist from Microbac. The same procedures were followed in performing the total bacteria counts, Staphylococcus aureus counts, and MRSA counts (see Laboratory Analysis of Pre-Existing Bacterial Contaminates, below). Zimek's Dri-Mist™ treatment was not performed on this date, therefore, no post-treatment set of samples were taken.

⁶ Source: Zimek's Treatment Status Software operating in the System

LABORATORY ANALYSIS: SWAB SAMPLES OF PRE-EXISTING BACTERIAL CONTAMINANTS

Four mL of Butterfield's buffered dilution water were pipetted into each sponge collection bag. Each collection bag was squeezed fifteen times to thoroughly disperse the collected bacteria in the liquid.

1. Total Plate Count of Bacteria

One mL of the liquid was removed from each sponge collection bag, under sterile conditions, and pipetted onto a Heterotrophic, Aerobic Petrifilm Count plate (ID #VEN0019-414-1, Expiration date 8/20/2008). These samples represented the 10^{-1} dilution. One-tenth of a mL of the liquid was pipetted onto a second Heterotrophic, Aerobic Petrifilm Count plate and nine-tenths of a mL of Butterfield's buffered dilution was added to each Petrifilm Count plate. This represented the 10^{-2} dilution. A one mL aliquot of liquid was removed from the sponge liquid and pipetted into a 99mL sterile Butterfield's dilution blank. This dilution blank was then capped and shaken for seven seconds at a 45 degree angle over a one foot arc. A one mL aliquot of this 1:1000 dilution was then pipetted onto a third Petrifilm Count plate. This represented the 10^{-3} dilution. The 72 Petrifilm Count plates (24x3) were then incubated with positive and negative controls for 48 hours at 35° C. Counts, of each red bacterial colony, were then taken on each Petrifilm plate and recorded in the laboratory notebook. As each sample stems from one-tenth of the bacteria in each swab, the laboratory counts are multiplied by ten in the report to represent the true count per swab. A 10^{-1} count of 24 results in a reported value of 240 CFU/swab. A count of zero results in a reported value of <10 CFU/swab. Standard approved procedures established by AOAC and FDA/BAM were used in the collection of bacteria and in their dilution onto Petrifilm plates.^{7&8}

2. Staphylococcus Aureus Bacteria Count

The 10^{-1} , 10^{-2} and 10^{-3} dilutions were prepared as in 1, above, for "Total Plate Count of Bacteria." These samples from the sponge liquid were each pipetted, under sterile conditions, onto a Staphylococcus Express Petrifilm Count plate (ID #VEN0019-438, Expiration date 5/15/2008). The 72 Petrifilm plates (24x3) were then incubated with positive and negative controls for 24 hours at 35° C. Counts were then taken of the violet colored Staphylococcus colonies. The Staphylococcus aureus film was then placed in those plates with positive Staphylococcus counts. After an additional three hour incubation period at 35° C, counts were made of those violet Staphylococcus colonies surrounded by pink rings (indicative of coagulase positive Staphylococcus aureus). These counts of Staphylococcus aureus were recorded in the laboratory notebook.

3. Methicillin Resistant Staphylococcus Aureus (MRSA) Bacteria Count

The sponge samples that had positive Staphylococcus counts were further tested for MRSA. The 10^{-1} , 10^{-2} and 10^{-3} dilutions were prepared as in paragraph 1., above. The samples from the sponge liquid were each pipetted, under sterile conditions, onto prepared Beckton Dickinson MRSA ChromAgar petri plates (Lot #7241186, Expiration date 11/13/2007). The liquid sample was spread with a sterile "hockey stick". These inoculated MRSA plates were incubated, inverted, for 24 hours at 35° C. The pink colored colonies were counted as MRSA bacteria and recorded in the laboratory notebook.

⁷ American Association of Analytical Chemists (AOAC) Official Method 966.23

⁸ Federal Department of Agriculture/Bacteriological Analytical Manual (FDA/BAM) Chapter 3

RESULTS

Based on the results of the Total Bacteria Count, Staphylococcus Aureus Count, and MRSA Count recorded in Microbac’s laboratory notebook, an individual data table and graph of these pre-existing bacterial contaminant levels were prepared, and average kill rates of contaminant levels were determined on each treatment area per swab tested, as follows:

RESULTS OF TOTAL PLATE COUNT OF BACTERIA⁹ (CFU/sponge) – 100 cm² of Surface Area

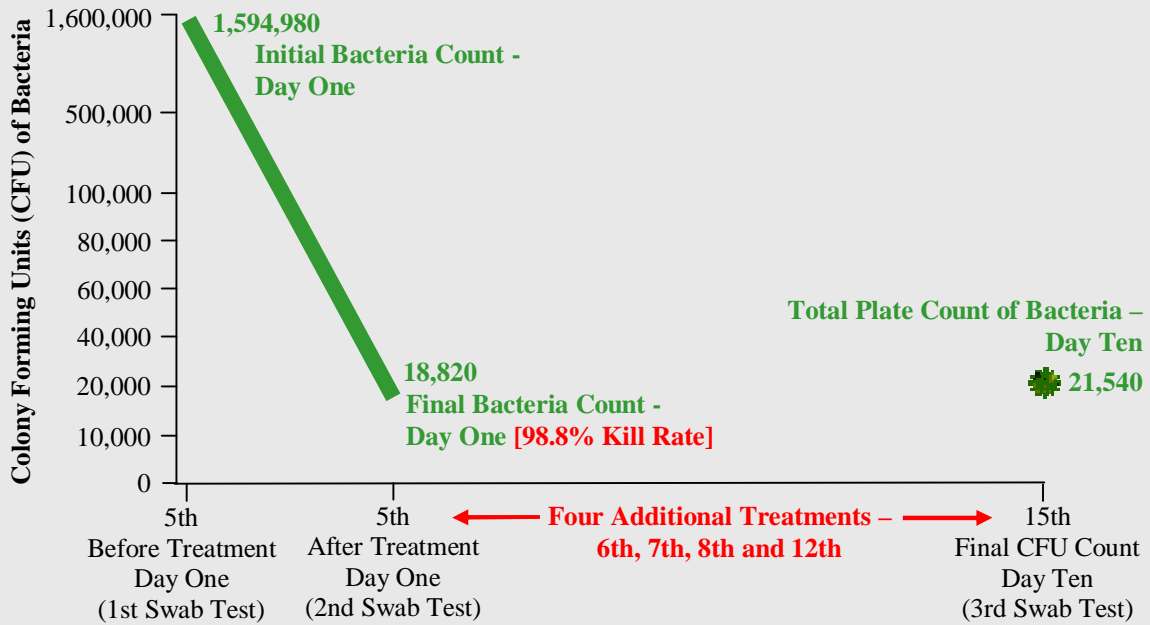
| Treatment Area | | 1 st Swab Test Before Treatment 09/05/07 | 2 nd Swab Test After Treatment 09/05/07 | Kill Rate % of CFU Killed between 1 st and 2 nd Swabs 09/05/07 | 3 rd Swab Test Follow-Up Study 09/15/07 | Kill Rate % of CFU Killed between 1 st and 3 rd Swabs 09/15/07 |
|--------------------------------------|------------------------------|--|--|--|--|--|
| 27 | Steering wheel | 100,000 | 2,700 | 97.3% | 4,200 | 95.8% |
| 28 | Floor (driver’s side) | 1,000,000 | 9,800 | 99.9% | 6,700 | 99.3% |
| 29 | Driver’s door handle area | 22,000 | 1,200 | 94.5% | 140 | 99.4% |
| 30 | Driver’s seat | 9,700 | 540 | 94.4% | 800 | 91.8% |
| 31 | Air conditioning return duct | 110,000 | 1,800 | 98.4% | 740 | 99.3% |
| 32 | Right side door handle | 10,000 | <10 | 99.9% | 20 | 99.8% |
| 33 | Needle drawer opening | 33,000 | 80 | 99.8% | 60 | 99.8% |
| 34 | Red EMT seat | 380 | 60 | 84.2% | 20 | 94.7% |
| 35 | Gear bag strap | 1,000 | 90 | 91.0% | 160 | 84.0% |
| 36 | Gurney lever | 97,000 | 540 | 99.4% | 1,500 | 98.5% |
| 37 | Ceiling by light | 1,900 | 10 | 99.5% | <10 | 99.9% |
| 38 | Fireman’s jacket | 210,000 | 2,000 | 99.0% | 7,200 | 96.6% |
| Total Plate Count of Bacteria | | 1,594,980 | 18,820 | | 21,540 | |
| Total Kill Rate of CFU | | | | 98.8% | | 98.6% |

<10

A count of zero results in a reported value of less than 10 CFU per swab (<10 CFU/swab).


⁹ CFU = “Colony Forming Units” of bacteria per specimen from an approximately four inch by four inch area located as noted.

RESULTS OF TOTAL PLATE COUNT OF BACTERIA



Before and After Dri-Mist™ Treatment Tests – September 2007

Note:


 The final Dri-Mist™ treatment was performed on September 12th for 10 minutes. The final swab test was performed on September 15th. In the interim period, the total plate count of bacteria increased due to replication of bacteria remaining on September 12th, or introduction of new bacteria after September 12th.

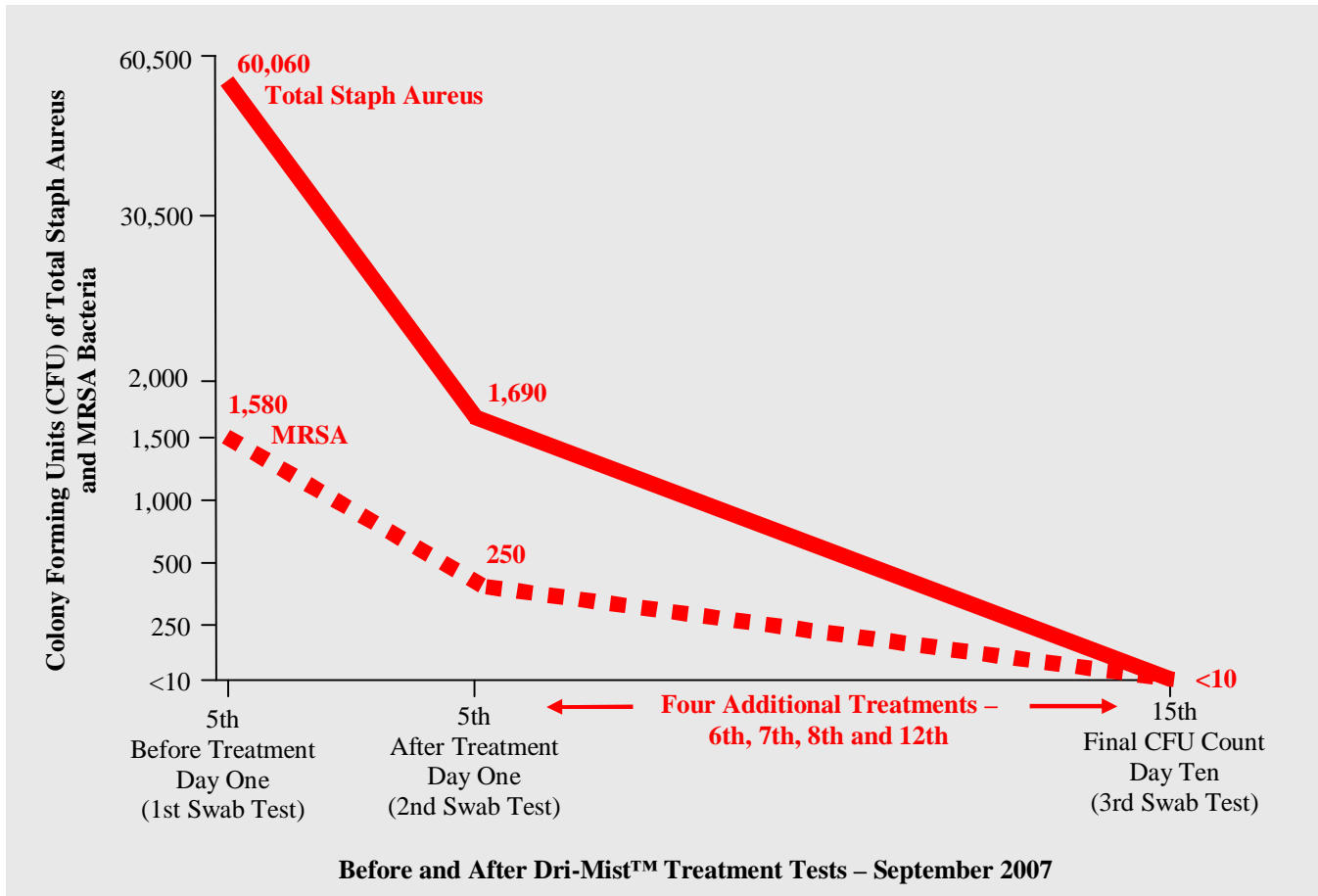
RESULTS OF TOTAL STAPHYLOCOCCUS AUREUS BACTERIA ONLY⁹
(CFU/sponge) – 100 cm² of Surface Area including MRSA Component on Select Surfaces

| Treatment Area | | 1st Swab Test Total Staph Aureus Before Treatment 09/05/07 | 2nd Swab Test Total Staph Aureus After Treatment 09/05/07 | Kill Rate % of Total Staph Aureus CFU Killed between 1st and 2nd Swabs 09/05/07 | 1st Swab Test MRSA Before Treatment 09/05/07 | 2nd Swab Test MRSA After Treatment 09/05/07 | Kill Rate % of MRSA CFU Killed between 1st and 2nd Swabs 09/05/07 | 3rd Swab Test Follow- Up Study 09/15/07 |
|-------------------------------|------------------------------|--|---|--|--|---|--|---|
| 27 | Steering wheel | 20,000 | 1,300 | 93.5% | 200 | <10 | 99.9% | <10 |
| 28 | Floor (driver's side) | 2,000 | 80 | 96.0% | - | - | - | <10 |
| 29 | Driver's door handle area | 2,500 | 110 | 95.6% | 280 | 50 | 82.1% | <10 |
| 30 | Driver's seat | <10 | <10 | - | - | - | - | <10 |
| 31 | Air conditioning return duct | 3,000 | 60 | 98.0% | - | - | - | <10 |
| 32 | Right side door handle | 2,800 | 40 | 98.6% | - | - | - | <10 |
| 33 | Needle drawer opening | 500 | <10 | 99.9% | - | - | - | <10 |
| 34 | Red EMT seat | <10 | <10 | - | - | - | - | <10 |
| 35 | Gear bag strap | 60 | <10 | 99.9% | - | - | - | <10 |
| 36 | Gurney lever | 200 | <10 | 99.9% | - | - | - | <10 |
| 37 | Ceiling by light | <10 | <10 | - | - | - | - | <10 |
| 38 | Fireman's jacket | 29,000 | 100 | 99.7% | 1,100 | 200 | 81.8% | <10 |
| Total Bacteria Count | | 60,060 | 1,690 | | 1,580 | 250 | | <10 |
| Total Kill Rate of CFU | | | | 97.2% | | | 84.2% | 99.9% |

<10

A count of zero results in a reported value of less than 10 CFU per swab (<10 CFU/swab).

RESULTS OF TOTAL STAPHYLOCOCCUS AUREUS BACTERIA INCLUDING MRSA



KEY FINDINGS

1. The total colony forming units (CFU) of all bacteria found in the areas tested in Fire Rescue Unit #16 and on the fireman's jacket was very significantly reduced by the 30 minute Dri-Mist™ treatment from 1,594,980 to 18,820, **resulting in a total bacteria kill rate on September 5th of 98.8%**.
2. The total Staphylococcus aureus bacteria remaining on September 15, 2007, including MRSA, was less than 10 (<10) CFU. Since the reported value of less than 10 (<10) CFU is counted as zero (0), **virtually all Staphylococcus aureus, including MRSA, was killed on:**
 - All tested areas in Fire Rescue Unit #16; and
 - The fireman's jacket.
3. The final Dri-Mist™ treatment prior to the September 15th swab test was administered three (3) days earlier on September 12th for ten minutes.⁶ Dri-Mist™ treatments did not occur on September 9th, 11th, 13th, 14th, and 15th. For this reason, increased plate count results were found on certain treatment areas on September 15, 2007 due to replication of remaining bacteria and/or introduction of new bacteria:

| Treatment Area | | 2 nd Swab Test After Treatment 09/05/07 | 3 rd Swab Test Follow-Up Study 09/15/07 | Increase of CFU between 2 nd and 3 rd Swabs 09/15/07 | % Increase of CFU between 2 nd and 3 rd Swabs 09/15/07 |
|-----------------------------|------------------------|---|--|---|--|
| 27 | Steering Wheel | 2,700 | 4,200 | 1,500 | 35.7% |
| 30 | Driver's seat | 540 | 800 | 260 | 32.5% |
| 32 | Right side door handle | <10 | 20 | 20 | 100.0% |
| 35 | Gear bag strap | 90 | 160 | 70 | 43.8% |
| 36 | Gurney lever | 540 | 1,500 | 960 | 64.0% |
| 38 | Fireman's jacket | 2,000 | 7,200 | 5,200 | 72.2% |
| Total Bacteria Count | | 5,870 | 13,880 | 8,010 | |

CONCLUSIONS

Despite being “cleaned” on a daily basis by fire rescue crews on duty, Fire Rescue Unit #16 (and the fireman’s jacket) had a very high total bacteria count prior to Zimek’s Dri-Mist™ treatment on September 5, 2007, including dangerous levels of infectious MRSA. The study proved that Zimek’s Dri-Mist™ treatment is very effective:

1. Dri-Mist™ killed virtually all Staphylococcus aureus bacteria, including MRSA, in both the vehicle and on the fireman’s jacket as evidenced by the swab test on September 15, 2007;
2. Dri-Mist™ is a dry application allowing reoccupation of the vehicle and re-use of the fireman’s jacket immediately after treatment; and
3. Regular applications of Dri-Mist™ will significantly eradicate MRSA thereby providing a healthier work environment for firefighters and paramedics, which will directly benefit their patients as well.

RECOMMENDATIONS

Due to the fact that bacteria replicate over time (see Findings 3, above), and fire rescue units and turnout gear are constantly subjected to dangerous contaminants like MRSA and Hepatitis C, it is imperative that Dri-Mist™ treatments be performed in fire rescue units and on turnout gear regularly, preferably every day. Dri-Mist™ treatments should also immediately be performed on fire rescue units and turnout gear whenever it is suspected that infectious pathogens have contaminated them, particularly if the infectious pathogens could be MRSA or Hepatitis C (Zimek’s QD disinfectant also kills Hepatitis C). It is notable that Zimek’s Dri-Mist™ applications are non-corrosive because they do not contain bleach or hydrogen peroxide (which are harmful oxidizing agents). Dri-Mist™ will not degrade turnout gear fabrics and should be used to kill bacteria and help prevent re-growth which will extend the life of turnout gear and protect firefighters, paramedics, and the patients they serve.

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