PPE Cleaning Validation

Verification of Cleaning, Decontamination, and Sanitization of Fire Fighter Garments

SUPPLEMENT H: Evaluation of Microbial Cleanliness of Selected ISP Advanced Cleaning Procedures

Jeffrey Stull
International Personnel Protection Inc.
Austin, Texas

September 2019
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Final Report by:
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September 2019
Fire fighter exposure to personal protective equipment (PPE) that is dirty, soiled, and contaminated is an increasing concern for long-term fire fighter health. Cancer and other diseases resulting from chronic exposures has become a leading issue and is presumed to be associated with fireground exposures relating to protection/hygiene practices and persistent harmful contamination found in fire fighter PPE.

While general cleaning procedures have been established in NFPA 1851, Standard on Selection, Care, and Maintenance of Protective Ensembles for Structural Fire Fighting and Proximity Fire Fighting, there are no requirements that demonstrate whether current cleaning practices will adequately remove contaminants from fire fighter PPE. Many manufacturer gear cleaning recommendations are vague and most cleaning product/process claims are unsubstantiated regarding contaminant removal effectiveness. Prior studies have identified persistent chemical and biological contaminants in structural firefighting PPE. Therefore, industry methodologies and practices are needed that can promote safe cleaning techniques so that fire fighters are not continually exposed to unclean or inadequately cleaned gear. It also important to set cleanliness criteria for the continued use of fire fighter protective clothing.

This project has established a relevant and credible procedure to validate “how clean is clean?” for fire service contaminated gear, and in doing so has addressed the primary goal of reducing fire fighter exposure to harmful contaminants in PPE. This includes the establishment of a repeatable and reproducible standardized method that can be used to determine the decontamination effectiveness of cleaning methods, and establish the needed fire service guidance for maintaining contaminant-free PPE as well as show that cleaning processes do not damage clothing. The project deliverables directly support efforts to update NFPA 1851 and other information that ensures consistent, effective cleaning processes of fire service gear.

This report is part nine of a nine-part series on this topic of “PPE Cleaning Validation”, with this part titled “Supplement H: Evaluation of Microbial Cleanliness of Selected ISP Advanced Cleaning Procedures”. The following are all the reports in this series:

1. Master Report
2. Supplement A: Annotated Bibliography
3. Supplement B: Preliminary Work for Assessing PPE Cleaning Procedures
4. Supplement C: Investigation of Simulated Fire Ground Exposures
5. Supplement D: Evaluation of Outer Shell Liquid Retention Properties
7. Supplement F: Report of Heavy Metals Contamination, Extraction, and Analysis Procedures
9. Supplement H: Evaluation of Microbial Cleanliness of Selected ISP Advanced Cleaning Procedures
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<th>Fire-Dex</th>
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<td>PBI Performance Products</td>
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<td>Lion First Responder Products</td>
<td>TenCate Protective Fabrics</td>
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<td>National Fire Protection Association</td>
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Keywords: PPE, Personal Protective Equipment, Fire Service, Fire Fighter, Contamination, Cleaning, Validation, Verification, Garments, Gear.

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# Table of Contents

- List of Figures 2
- List of Tables 3
- Abstract 4
- Approach 4
- Results 8
- Analysis and Discussion 10
- Additional Evaluation of Microbial Cleanliness at Single ISP Facility 13
- Overall Conclusions 14
- Appendix A: Instructions for ISP Facility Microbial Cleanliness Study 15
List of Figures

Figure 1: ISP C selected swatch locations for coat shell exterior 5
Figure 2: ISP C selected swatch locations for coat shell interior 5
Figure 3: Swatch being affixed to interior of pant shell at ISP D 6
Figure 4: Affixed swatch on coat shell sample at ISP D 6
Figure 5: General bacteria counts by ISP and garment swatch location 9
Figure 6: TSA plates (general bacteria) from ISP C sample set 11
Figure 7: Additional plates with isolation of yeasts from ISP C sample set 11
Figure 8: Mold growth on an MSA plate from ISP D 12
Figure 9: Plating to identify bacillus on swatch from ISP E 12
Figure 10: Relative resistance of microorganisms to sanitization and disinfections 13
List of Tables

Table 1: Advanced Cleaning Parameters and Procedures 7
Table 2: Types of Bacteria Evaluated in Selective Analyses 8
Table 3: General Bacteria Counts by Swatch for Each ISP 9
Table 4: Counts for Selective Bacterial by ISP and Swatch 10
Table 5: Bacteria and Fungi Counts by Process Type and Swatch 14
Abstract

A small-scale study was carried out to determine the microbial cleanliness of the facilities for selected independent service providers (ISPs) that provide commercial laundering and sanitization services of turnout clothing for fire departments. This testing was undertaken because the application of proposed verification procedures for sanitization of fire fighter protective clothing samples yielded different types of bacteria other than the bacteria used in the inoculation of test samples. Background microbial counts were considered important because high microbial counts sometimes interfere with techniques applied to determine the effectiveness of sanitizing agents or processes on contaminated samples that are transported to ISPs for sanitization and advanced cleaning.

This testing was carried out at five different ISPs and involved procedures to determine the general types and amounts of bacteria that were present at each facility during processes used for advanced cleaning of fire fighter turnout clothing. The testing was performed by sending sterilized swatches of turnout clothing outer shell materials to each ISP and having the ISP aseptically pin the swatches onto the exterior and interior of standard turnout clothing and then put the gear through their normal advanced cleaning procedures. Following cleaning, the swatches were returned to a microbiology laboratory where they were analyzed for counts of bacteria on each individual swatch. In general, the testing showed minimal or manageable levels of bacteria for most of the returned specimens, although there were differences between ISPs that could be considered significant. An analysis of the samples for specific targeted pathogenic bacteria revealed only a couple of cases where such bacteria were found.

A separate study was conducted with one of the original ISPs to examine differences for microbial cleanliness involving three different processes for handling the samples, which also included evaluating levels of fungal microorganisms that had been observed but not quantified in the first set of testing. Based on this testing, distinct differences were observed for three cleaning and handling procedures evaluated.

Overall, this testing pointed to the value of maintaining “clean” conditions in the sanitization of fire fighter protective clothing for avoiding other forms of microbial contamination. ISPs participating in the process undertook specific steps to improve the cleanliness of their facilities in response to learning the results of this testing.

Approach

Five different ISPs were identified in different parts of the United States that actively engage in the cleaning of fire fighter turnout clothing for fireground soiling and biological contamination as may occur for clothing that is contaminated with blood/body fluid or other forms of biological contaminants. These companies included one organization that participated in the assessment of sanitization effectiveness and four other organizations that had not been previously evaluated. Each of the organizations is identified by a letter code (A through E).

Swatches of an outer shell material (identified in the project as Material A) that had been subjected to 10 cycles of laundering per AATCC 135 were sterilized in an autoclave and then sent to each ISP with instructions to affix the swatches to a set of turnout coat. A copy of these instructions are provided in Appendix A. The ISPs were further instructed to apply aseptic procedures (use sterile safety pins, examination gloves, tweezers, alcohol wipes, and sterile return containers) in placing the swatches on coat and pant outer shells and to put the items of clothing through on cycle of an advanced cleaning process. ISPs were told to use a clean, relatively new set of coat and pant outer shells for this purpose.

Eight different swatches were specified for placement on the garments where:

- Swatches #1 and #2 were to be affixed to the exterior of the coat outer shell
- Swatches #3 and #4 were to be affixed to the interior of the coat outer shell
- Swatches #5 and #6 were to be affixed to the exterior of the pants outer shell
- Swatches #7 and #8 were to be affixed to the interior of the coat outer shell

The swatches were also identified by small pieces of sterile paper affixed the swatch. Garment liners were not addressed because the initial procedures for sanitization of garments only addressed outer shells.

Each ISP had the latitude to select the swatch location but were instructed to indicate the positions where the swatches were placed on diagrams supplied with the instructions. Figures 1 and 2 show an example where one of the ISPs affixed their swatches to a protective coat outer shell. Photographs of the actual swatch affixed to sample garments are provided in Figures 3 and 4.
Figure 1: ISP C selected swatch locations for coat shell exterior

Figure 2: ISP C selected swatch locations for coat shell interior
Supplement H: Evaluation of Microbial Cleanliness of Selected ISP Advanced Cleaning Procedures

Figure 3: Swatch being affixed to interior of pant shell at ISP D

Figure 4: Affixed swatch on coat shell sample at ISP D
The ISPs were then told to apply their normal advanced cleaning procedures for handling the respective garments but note the general details of their procedures. These advanced cleaning details are documented in Table 1.

<table>
<thead>
<tr>
<th>ISP</th>
<th>Type of Work Surface*</th>
<th>Coat Mfg. Date/Condition</th>
<th>Pant Mfg. Date/Condition</th>
<th>Washing Equipment and Procedures</th>
<th>Drying Equipment and Procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Formica countertop</td>
<td>No date, spare gear, previously serviced</td>
<td>No date, spare gear, previously serviced</td>
<td>60 lb. washer/extractor, 12 min. warm wash cycle with proprietary detergent, 3 rinse cycles cold water; washed with 2 other sets</td>
<td>60 lb. dryer at no heat setting, 30 min.</td>
</tr>
<tr>
<td>B</td>
<td>Stainless steel table</td>
<td>2011 used, normal use, previously washed</td>
<td>2011 used, normal use, previously washed</td>
<td>65 lb. washer/extractor, with prewash flush, suds cycle, and 4 rinses; washed at 80% load with other garment outer shells</td>
<td>Air dry in room with conditioned air, fans, and dehumidifier</td>
</tr>
<tr>
<td>C</td>
<td>Formica countertop</td>
<td>2008, good condition</td>
<td>2008, good condition</td>
<td>60 lb. washer/extractor; 15 min. wash warm water &lt;105°F; 3 cold rinses (7 min. each) extract 6 min; use of proprietary detergent; washed with 6 other garment outer shells</td>
<td>70 lbs. tumble dryer 20 min. &lt;105°F</td>
</tr>
<tr>
<td>D</td>
<td>Stainless steel table</td>
<td>2012, like new, previously washed several times</td>
<td>2012, like new, previously washed several times</td>
<td>55 lb. washer/extractor; 2 suds cycles followed by 3 rinse cycles, each with ozone; washed with 5 other garment outer shells</td>
<td>Air dry</td>
</tr>
<tr>
<td>E</td>
<td>Stainless steel table</td>
<td>2013, used, spare gear, previously washed</td>
<td>2013, used, spare gear, previously washed</td>
<td>60 lb. washer/extractor, warm wash, 3 rinse cycles, d-Limonene based detergent; with five other garment outer shells</td>
<td>Air dry in conditioned space with fans</td>
</tr>
</tbody>
</table>

* Used for handling of gear for affixing sterile swatches

Following laundering, the washed swatches were aseptically removed from the clothing and transferred to sterile containers that were then sent back to the microbiological laboratory. Each set of samples were then subjected to standardized microbiological plating procedures for counting the bacteria on each sample. Swatches were vortex mixed in 20 mL of Dey/Engley neutralizing broth for collection of bacteria followed by one of the following procedures depending on the type of bacterial analysis. The following analysis were performed:

- The total number of bacteria present was determined by plating on Tryptic Soy Agar (TSA) nutrient media with −1, −2, and −3 serial dilutions. Full quantitative plates were incubated for ≥ 5 days at 30 ± 2°C after which, plates were read with microbiological colony counting techniques; these techniques had a lower detection limit of 10 colony forming units (CFU) per swatch.
- The analysis selective for Staphylococcus bacteria involved plating on Mannitol Salt Agar nutrient media.
- The analysis selective for Coliform Bacteria involved plating on M-Endo agar nutrient media.
- The analysis selective for Pseudomonas bacteria involved plating on Pseudomonas Isolation Agar (PIA) media.
- M-Endo, MSA, and PIA plates were incubated for ≥ 2 days at 36°C ± 1°C. All of the special bacterial analyses has a lower detection limit of 20 CFU/sample.

Special bacterial analyses were performed because the selected bacterial genera include known pathogenic microorganisms as shown in Table 2. All analyses were performed by a qualified microbiological laboratory accredited to ISO 17025, General Requirements for the Competence of Testing and Calibration Laboratories. Individual reports were issued to each participating ISP.
Table 2: Types of Bacteria Evaluated in Selective Analyses

<table>
<thead>
<tr>
<th>Type of Bacteria</th>
<th>Representative Species</th>
<th>Photograph</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genus Staphylococcus—affect skin and mucous membranes; associated with contaminated food and hospital-based infections</td>
<td><em>S. aureus</em> (includes MRSA) common cause of skin infections including abscesses, respiratory infections such as sinusitis, and food poisoning. Pathogenic strains often promote infections by producing virulence factors such as potent protein toxins.</td>
<td><img src="image" alt="Staphylococcus aureus" /></td>
</tr>
<tr>
<td>Coliform bacteria are organisms that are present in the environment and in the feces of all warm-blooded animals and humans*</td>
<td><em>Escherichia coli</em> commonly found in the lower intestine of warm-blooded organism</td>
<td><img src="image" alt="Escherichia coli" /></td>
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<tr>
<td>Genus Pseudomonas—rod-shaped, gram-negative, aerobic, non-spore forming bacteria found in animals, plants, and soil</td>
<td><em>P. aeruginosa</em> flourishes in hospital environments, and is a particular problem in this environment, since it is the second-most common infection in hospitalized patients</td>
<td><img src="image" alt="Pseudomonas aeruginosa" /></td>
</tr>
</tbody>
</table>

* Coliform bacteria will not likely cause illness. However, their presence in drinking water indicates that disease-causing organisms (pathogens) could be in the water system.

Results

Bacterial counts were provided for the number of colony forming units per individual sample (CFU/sample). These results are provided in Table 3 and graphically shown in Figure 5. The results equate to the total number of respective bacteria found per swatch and appear rounded because the raw results were reported in an exponent format with three significant figures (e.g., 1.23E + 04 is 1,230). The average bacterial count by ISP was also determined. Similar results for the analysis of selective bacteria on each of the swatches from each ISP are given in Table 4.
Table 3: General Bacteria Counts by Swatch for Each ISP

<table>
<thead>
<tr>
<th>ISP</th>
<th>Swatch Number</th>
<th>Avg.</th>
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<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>A</td>
<td>70</td>
<td>500</td>
</tr>
<tr>
<td>B</td>
<td>70</td>
<td>300</td>
</tr>
<tr>
<td>C</td>
<td>890</td>
<td>50</td>
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<tr>
<td>D</td>
<td>50</td>
<td>80</td>
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<tr>
<td>E</td>
<td>40</td>
<td>20</td>
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BDL = Below detection limit (<10 CFU/sample)

Figure 5: General bacteria counts by ISP and garment swatch location
Table 4: Counts for Selective Bacteria by ISP and Swatch

<table>
<thead>
<tr>
<th>ISP</th>
<th>Swatch Number</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<th>5</th>
<th>6</th>
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<tr>
<td>Genus Staphylococcus</td>
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<td>Coliform Bacteria</td>
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<td>Genus Pseudomonas</td>
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BDL = Below detection limit (< 20 CFU/sample)

Analysis and Discussion

Based on these results, there were differences in the bacterial levels among the different ISPs evaluated with two different ISPs reporting bacteria counts one order of magnitude lower than the three other ISPs. Based on the experience of the microbiological laboratory conducting this testing, bacteria counts numbering ~300 bacteria colonies per sample are compared to results received on hospital surfaces that have undergone cleaning and sanitizing. Therefore, swatches with bacterial counts above 500 or 1000 would suggest lower levels of microbial cleanliness, which could be rectified by the application of more sanitized handling procedures and surfaces. It was further pointed out that most clothing worn in everyday life will have higher bacteria counts than those reported here.

There was no discernible pattern by swatch location or if the swatch was placed on either the exterior or interior of the garment outer shell. Total bacteria levels varied within the garment swatch location, with the widest ranges for those ISPs with higher bacterial counts.

Staphylococcus and Pseudomonas bacteria were identified at the detection limit for one swatch each at two different ISPs. A third ISP showed just Pseudomonas bacteria also at the detection limit on one swatch. No Coliform bacteria were found on any swatches. These levels are considered relatively low and not indicative of any specific pathogenic bacterial issues at any of the evaluated ISP locations. According to the microbiological laboratory involved in this testing, any pathogenic colony count that was found for these swatches would be considered too low to cause infection.

While analyses of the laundering subjected samples were focused on bacterial contamination, additional microorganisms were found to be present and other observations were made based on the application of microbiological analysis of the returned samples. Generalized findings from these observations are provided in the attached report. These included:

- The majority of bacteria found on samples were determined to be of the Genus Bacillus.
- Various type of fungi were observed in growing cultures from each of the samples. Both yeasts (single-cell fungi) and molds (multiple-cell fungi) were seen.

Several examples of the plating results are shown in Figures 6 through 9. In Figure 6, the yellow growth on Plate C3 and the red/pink growth found on Plate C5 were found through culture isolation and inspection with a microscope to be fungal in
nature. Growth seen on the other plates was cultured and isolated, inspected with a microscope, and determined to be *Bacillus*. Figure 7 shows fungal cultures separately isolated from the same C1 to C8 plates. These fungal growths were determined to be yeasts when examined under magnification. Figure 8 shows mold growth (fungus) on an MSA plate from ISP D that was meant to isolate *Staphylococcus aureus* colonies. Bacillus isolated from an ISP E test sample is pictured in Figure 9.

**Figure 6:** TSA plates (general bacteria) from ISP C sample set

**Figure 7:** Additional plates with isolation of yeasts from ISP C sample set
Figure 8: Mold growth on an MSA plate from ISP D

Figure 9: Plating to identify bacillus on swatch from ISP E
The finding of *Bacillus*-based bacteria is significant. Ubiquitous in nature, *Bacillus* includes both free-living (nonparasitic) and parasitic pathogenic species. Under stressful environmental conditions, the bacteria can produce oval endospores that are not true ‘spores’, but to which the bacteria can reduce themselves and remain in a dormant state for very long periods. In general, the nature of *Bacillus*-based bacteria makes sanitization and disinfection difficult. In the hierarchy of resistance to anti-microbial treatments, spore-forming bacteria are among the most difficult microorganisms to kill, as shown in Figure 10.

**Figure 10:** Relative resistance of microorganisms to sanitization and disinfections

<table>
<thead>
<tr>
<th>Resistance to Germicidal Chemicals</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacterial Spores:</strong> <em>Bacillus</em> species, <em>Clostridium</em> species</td>
</tr>
<tr>
<td><strong>Mycobacteria:</strong> <em>Mycobacterium tuberculosis</em></td>
</tr>
<tr>
<td><strong>Nonlipid or Small Viruses:</strong> Poliovirus, Coxsackievirus, Rhinovirus</td>
</tr>
<tr>
<td><strong>Fungi:</strong> <em>Trichophyton</em>, <em>Cryptococcus</em>, <em>Candida</em> species</td>
</tr>
<tr>
<td><strong>Vegetative Bacteria:</strong> <em>Pseudomonas aeruginosa</em>, <em>Staphylococcus aureus</em>, <em>Salmonella choleraesuis</em>, Enterococci</td>
</tr>
<tr>
<td><strong>Lipid or Medium-size Viruses:</strong> Herpes simplex virus, Hepatitis B, Hepatitis C, Human immunodeficiency virus (HIV), Hantavirus, Ebola virus</td>
</tr>
</tbody>
</table>

The presence of fungi are not in themselves dangerous; however, large numbers of fungi can overwhelm the ability to identify and count target bacteria when used as a means for assessing the effectiveness of sanitization and disinfection processes. Therefore, an understanding of their persistence and sources can be helpful for improving the evaluation of sanitization and disinfection effectiveness.

**Additional Evaluation of Microbial Cleanliness at Single ISP Facility**

Further testing was conducted at ISP D to ascertain whether different handling practices would affect the microbial cleanliness for handling of fire fighter clothing for purposes of sanitization. In this effort, the same procedures were applied with the use of sterile swatches, the aseptic positioning of those swatches at 8 different locations on different sets of fire fighter clothing outer shells, subjecting the specified advanced cleaning with different handling and process techniques, and evaluating the swatches for levels of microbial contamination.

One additional analysis was conducted for this follow-on testing that included the determination of fungal levels. This was undertaken by plating the vortexed solution from the swatches onto Potato Dextrose Agar (PDA) and then continuing serial dilutions as needed for counting fungal microorganisms present.

Three different laundering and handling techniques were evaluated that were based off the general laundering procedures that were applied in the first set of procedures. Those procedures included the 55 lb. washer/extractor with 2 wash cycles followed by 3 rinse cycles where the swatch-based outer shell clothing was combined with 5 other garment outer shells. Specific variants in these procedures involved:

- Injection of ozone into the rinse cycles with no drying (swatches returned wet)
- Injection of ozone into the rinse cycles with overnight drying in drying room
- Machine washing with addition of a commercial sanitizer (no ozone was used in rinsing) with overnight drying in drying room
For this testing, no detectable bacteria were found in the bacteria selective tests (Genus Staphylococcus, Coliform bacteria, and Genus Pseudomonas). Table 5 provides the test results for total counts for bacterial and fungal microorganisms by swatch for each of the three applied processes.

Table 5: Bacteria and Fungi Counts by Process Type and Swatch

<table>
<thead>
<tr>
<th>Process Type</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria (total)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ozone/Wet</td>
<td>15,500</td>
<td>19,900</td>
<td>11,100</td>
<td>7,600</td>
<td>23,400</td>
<td>19,700</td>
<td>30,500</td>
<td>12,700</td>
</tr>
<tr>
<td>Ozone/Dry</td>
<td>380</td>
<td>2,140</td>
<td>BDL</td>
<td>20</td>
<td>60</td>
<td>240</td>
<td>280</td>
<td>20</td>
</tr>
<tr>
<td>Sanitizer/Dry</td>
<td>400</td>
<td>100</td>
<td>20</td>
<td>BDL</td>
<td>640</td>
<td>BDL</td>
<td>20</td>
<td>20</td>
</tr>
</tbody>
</table>

| Fungi (total) |       |       |       |       |       |       |       |       |
| Ozone/Wet    | 14,200| 18,700| 9,700 | 5,300 | 22,100| 10,500| 34,400| 2,800 |
| Ozone/Dry    | 40    | 140   | 20    | 40    | 40    | BDL   | 20    | 60    |
| Sanitizer/Dry| 80    | 40    | 20    | BDL   | 100   | 20    | 20    | 40    |

These results showed overwhelmingly the high microbial contamination of the swatches that occurs when left wet without adequate drying when transported wet. The use of a sanitizer further provided slightly lower bacterial and fungi counts as compared to using ozone injection during the rinse cycles. This testing provided the ability to discriminate facility microbial cleanliness using different procedures.

Overall Conclusions

Since fire fighter protective clothing frequently becomes contaminated with blood, body fluids, and other biological contaminants, it is necessary to undertake sanitization to minimize the hazards of any pathogenic microorganisms that may be present as a result of these exposures. NFPA 1851 has been updated in its 2020 edition to specify sanitization of fire fighter protective clothing using appropriate procedures. Manufacturers of this clothing, as well as independent service providers (ISPs), are required to verify the effectiveness of their sanitization procedures using modified EPA-based test methods for samples inoculated with target bacteria. These samples are then subjected to the sanitization procedures used at the specific manufacturer ISP facility and are returned to a qualified laboratory to ascertain the procedure effectiveness for neutralizing target bacteria. However, if other forms of microbial contamination are present at the ISP or manufacturer facilities, then the ability to ascertain the effectiveness for sanitization procedures can be hampered. Further, the application of sanitization should imply that the majority of microorganisms have been removed, even if under nonsterile conditions.

This study was undertaken to gain an understanding for the general microbial cleanliness at selected ISPs. The use of sterile swatches of outer shell material subjected to advanced cleaning procedures at different ISPs generally showed acceptable levels of background microbial contamination, but also could indicate the need for better control of working surfaces and handling of cleaned turnout clothing. For the most part, background microbial levels for general bacteria are similar to those found on hospital surfaces that have been subjected to cleaning and sanitization. Further, there was a general absence of any pathogenic bacteria that could be considered a concern if there was an abundance of biological contamination at facilities handling and cleaning turnout clothing.

The study was further able to show the ability to discriminate variations in advanced cleaning and sanitization techniques as applied in an individual ISP. Therefore, the general approach for assessing microbial cleanliness can be used as a tool for understanding sources and remedies for achieving higher levels of sanitization.
Appendix A: Instructions for ISP Facility Microbial Cleanliness Study

Objective

To determine the number and different types of bacteria that may be found at selected ISP turnout clothing cleaning facilities.

Background

Prior work by the Fire Protection Research Foundation for evaluating the effectiveness of sanitization approaches by different independent service providers (ISPs) has shown that while the test procedures can determine the reduction of bacterial contamination, foreign bacteria can sometimes interfere with the ability to determine biological decontamination. Normally, sterile fabric swatches are contaminated with a specific form of target bacteria that are then inserted into the wash/sanitization process at the ISP. The return of sanitized samples can provide an indication for the ability of the ISP process for reducing bacterial counts. However, while sanitizers can be effective in removing target bacteria, other bacteria may be present from a variety of sources at the ISP or through handling that can contaminate sanitized fabric swatches during and after processing. Therefore, there is a need to understand the different types of bacteria that may be present at ISPs so that test laboratories can better discern the results of verification testing.

Approach

Each ISP will be provided with 8 sterilized outer shell specimens. Each specimen measures approximately 2.5” × 2.5” and is numbered 1 through 8. The ISP will affix the specimens onto both a coat and pants outer shell and put them to ordinary advanced cleaning as part of a normal wash load. Following the advanced cleaning, half of the specimens (4) will be removed while the remaining specimens stay on the outer shell items and are subjected to ordinary drying. All specimens will be returned to the laboratory where they will be evaluated for number and types of bacteria. It is important that personnel handling the specimens use techniques to prevent cross contamination of the specimens when they are put on and taken off the clothing. Specific procedures are described below.

Instructions

1. Choose a coat and pant outer shell to serve as the clothing items for fixing the sterile outer shell specimens.
2. Identify a clean working surface for manipulating the clothing and fixing the sterile specimens.
3. Have disposable examination gloves and alcohol based wipes available for handling specimens.
4. Open the box provided by Microchem Laboratory.
5. Remove the individual packages containing the sterilized outer shell specimens and safety pins.
6. Select 2 locations on both the exterior and interior of the coat outer shell for placement of one outer shell specimen each. Use specimens 1 and 2 for the exterior and specimens 3 and 4 for the interior.
7. Using an individual safety pin, and while wearing examination gloves, affix an individual outer shell specimen at each of the selected locations on the coat shell. Wipe glove finger tips with an alcohol wipe after handling each specimen.
8. Take a photograph or make a sketch where each outer shell specimen is located on the coat shell (diagrams are attached for marking locations).
9. Similarly, select 2 locations on both the exterior and interior of the pant outer shell for placement of one outer shell specimen each. Use specimens 5 and 6 for the exterior and specimens 7 and 8 for the interior.

10. Using an individual safety pin, and while wearing examination gloves, affix an individual outer shell specimen at each of the selected locations on the pant shell. Wipe glove finger tips with an alcohol wipe after handling each specimen.

11. Take a photograph or make a sketch where each outer shell specimen is located on the pant shell.

12. Place both coat and pant shells with a fixed outer shell specimen in with a normal load of clothing for advanced cleaning.

13. Subject the load to advanced cleaning using the normal procedures.

14. Following the advanced cleaning, remove the coat and pant shell with affixed specimens.

15. Using examination gloves, remove specimens 1, 3, 5, and 7 from the coat and pant shells and place each specimen into their original container. Wipe glove finger tips with an alcohol wipe after handling each specimen. The safety pins can be discarded.

16. Put the coat and pant shell back in with the other clothing being cleaned and subject to drying using your ordinary drying procedures.

17. At the end of the drying period, remove specimens 2, 4, 6, and 8 from the coat and pant shells and place each specimen into their original container. Wipe glove finger tips with an alcohol wipe after handling each specimen. The safety pins can be discarded.

18. After all of the specimens have been recovered, ship the specimens by overnight service back to the microbiological laboratory.

**Follow Up**

Results will be separately provided to you following their analysis. All overall results will be coded.
Specimen Locations—Coat Shell Exterior

Front

Back
Specimen Locations—Coat Shell Interior

Front

Back
Specimen Locations—Pant Shell Exterior

Front

Back
Specimen Locations—Pant Shell Interior