Biocontamination Control for Space Suit Garments – A Preliminary Study

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This paper outlines a preliminary study that was conducted to review, test, and improve on current space suit biocontamination control. Biocontamination from crew members can cause space suit damage and objectionable odors and lead to crew member health hazards. An understanding of the level of biocontamination is necessary to mitigate its effects. A series of tests were conducted with the intent of evaluating current suit materials, ground and on-orbit disinfectants, and potential commercial off-the-shelf antimicrobial materials. Included in this paper is a discussion of the test methodology, results, and analysis method.

Nomenclature

ACES = Advanced Crew Escape Suit
BladderG = ACES GORE-TEX™ bladder
BladderU = EMU polyurethane-coated nylon bladder
CFU = colony-forming unit
COTS = commercial off-the-shelf
CSSE = Constellation Space Suit Element
CxP = Constellation Program
EMU = EVA Mobility Unit
EVA = extravehicular activity
HPC = heterotrophic plate count
LCG = liquid cooling garment
LCVG = liquid cooling ventilation garment
LCVG-L = LCVG-inner layer
LCVG-O = LCVG-outer layer
PA = polyamide
PBS = phosphate buffer saline
PET = polyester (polyethylene terephthalate)
PGS = pressure garment suit
SDA = Sabouraud dextrose agar
TCU = thermal comfort undergarment

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I. Introduction

This paper outlines a preliminary study to understand the current state of space suit biocontamination control. The study includes an evaluation of current and advanced space suit materials, ground and on-orbit cleaning methods, and microbial test and analysis methods. Biocontamination from crew members during extravehicular activities (EVAs) can lead to suit damage, odors, and crew member health hazards. It is advantageous to reduce the level of biocontamination on a space suit to lessen this risk.[1]

The first stage of this study was to identify potential antimicrobial textiles and cleaning agents. The antimicrobial cleaning agent and textile market survey focused on current commercial off-the-shelf (COTS) products that could potentially be used as future space suit materials, replacing any currently used soft-good layers that may become contaminated during an EVA, including the pressure bladder, liquid cooling garment (LCG), and ancillary thermal comfort undergarment (TCU).

The second stage of this study was to review standardized test procedures (AATCC, ASTM, etc.) to evaluate how current and advanced materials could be evaluated. A customized test procedure, developed after consideration, is discussed within this paper.

Finally, four tests were conducted to evaluate current and COTS materials and cleaning agents: (1) a test of the stacked layers arrangement of current suit materials to understand how biocontamination propagates through the various suit layers; (2) a test of each current suit material layer to evaluate the efficacy of each soft-good layer to repress microbial growth; (3) an efficacy test for the suppression of microbial growth by cleaning agents on each of the current suit bladder materials; and (4) a test evaluation of the efficacy of various COTS antimicrobial textiles to suppress microbial growth.

All antimicrobial COTS materials tested appeared to control bacterial colony-forming unit (CFU) growth better than the TCU and the Advanced Crew Escape Suit (ACES) LCG/Extravehicular Activity Mobility Unit (EMU) liquid cooling ventilation garment (LCVG). However, a comparison of fungal CFU growth in COTS to current suit materials appeared to vary with material. The EMU polyurethane-coated nylon bladder also seems to be more responsive to cleaning than the ACES GORE-TEX™ (W. L. Gore and Associates, Inc., Newark, DE). Other trends and a series of test improvements for future microbial testing are discussed below.

II. Study Methodology

A. Evaluation of the Problem

Prolonged microbial growth in a space suit presents hygienic and functional risks, including foul odors rendering the suit unusable, health hazards, and operational risks due to textile degradation. These risks must be evaluated as the U.S. human space program moves toward planned long-term missions beyond low Earth orbit (LEO). Thus, there is a need for a comprehensive, systematic study on suit biocontamination. Previous studies have evaluated the frequency of cleaning and its influence on the life of suit components,[1] but no extensive research has been undertaken on the use of different textile materials and technologies to control microbial growth.

As NASA continues to shift its focus from LEO to exploration beyond LEO, the effects of biocontamination become a greater concern for the health of the suit and the crew members. This concern was also made evident in the recent Constellation Program's requirements. The Constellation Program (CxP) operations concept included confined and limited habitable spaces in which the space suit is used as many as 90 times over 6 months for lunar missions, and stored as many as 210 days on Orion-based missions to the International Space Station or other near-Earth-orbit destinations.

The specification used in this study for microbial contamination level limitation is provided in Reference 2. This document was used to write the requirements for acceptable Constellation space suit element (CSSE) biocontamination levels, as listed in Reference 3. The two applicable requirements from the Element Requirement Document (ERD, Reference 3) are:

1. *[CSSE1105] Microbial Contamination*

The pressure garment suit (PGS) shall limit the level of microbial contamination on the interior surface of the bladder to 4×10^6 CFU/100 cm^2 or fewer following the post-doff cleanup procedure and subsequent storage for up to 210 days.

2. *[CSSE1142] Fungal Contamination*

The PGS shall limit the level of fungal contamination on the interior surface of the bladder to 100 CFU/100 cm^2 or fewer following the post-doff cleanup procedure and subsequent storage for up to 210 days.

B. Antimicrobial Materials Selection

As a wealth of textile fabrics and antimicrobial technologies exist, the challenge in material selection is to identify the most likely textile candidates for in-suit materials. This section provides background information on the different types of anti-microbial materials and describes the selection process that was used for this test.

Textiles made of natural fibers can discolor or rot from microbial attack, especially if they are kept in hot and humid conditions. Synthetic fibers are inherently resistant to microbial attack and to decomposition, but are good substrates for microbial growth, especially those used for lingerie and undergarments.

The sportswear, underwear, and shoe-lining industries are now selling a variety of antimicrobial textiles. These new textiles have incorporated active antimicrobial agents such as silver, copper, quaternary ammonium salts, polyhexamethylene biguanide, organo-silanes, triclosan, chitosan, dyes, and regenerable *N*-halamine compounds and peroxyacids. Although antimicrobial agents are divided into biocides and biostats, biocides account for most of the market growth. Antimicrobial agents are attached to the fabric surface or incorporated within the fiber. As their name suggests, they can stop microbial growth by inhibition or destruction of the microorganisms through one or more of the following mechanisms: cell wall damage, inhibition of cell wall synthesis, alteration of cell wall permeability, inhibition of the synthesis of proteins and nucleic acids, and/or inhibition of enzyme action. The biocidal agents used in the textile industry can be divided in two general categories: those used for cellulosic or synthetic fibers. Chitosan, *N*-halamine, and peroxyacids are usually bonded to cellulosic cotton, whereas metals are added to synthetic fibers. These agents can also be divided into leaching and affixed agents.

Leaching agents are disadvantageous because they create a zone of inhibition with a concentration gradient from the source to the edge. This means that a sublethal effect is likely to be found with these types of antimicrobial agents, and new generations of resistant microbes may be selected in the process.

Affixed agents bond to fibers or fabrics. Most antimicrobial textiles contain these agents, which work by physically and ionically attacking microbes. Metal ions and oxides belong to this category.

Currently, silver and copper are the most commonly used antibacterial additives in textiles. Both silver and copper host a number of advantages: they have a high degree of biocompatibility, excellent resistance to sterilization, and broad spectrum antibacterial properties.

The selection of antimicrobial fabric samples in this study was based on the following requirements:

- Use with synthetic fibers for space suit fabrics
- Durability to washing, dry cleaning, hot pressing
- Broad biocidal effect (gram-positive and gram-negative bacteria, and fungi) and non-leaching
- Compliance with statutory requirements in terms of toxicity and environmental impact
- Resistance to sterilization
- Market availability (penetration into the performance apparel market as in the number of products with silver-nanoparticle-impregnated fibers)

Table 1. Commercial Off-the-shelf Antimicrobial Fabric Candidates

Product Identification	Materials	Material Content by % Weight
Cupron™* NASA-2	PET/copper oxide	98/2
Cupron™ NASA-4	PET/copper oxide	98/2
Cupron™ NASA-5	PET/copper oxide	98/2
UNITED KNITTING† 80031	PET/PA/Ag ion	92/7/1
UNITED KNITTING 65961	PET/PA/Ag ion/spandex	20/58/8/14
KAOS**	PA/Ag ion/spandex	79/11/10
SILVERCLEAR®††	PA/Ag chloride	Unknown
HYGY™ *** H3 TTM175-80 MA	PET/silicone derivative	Unknown

*Cupron, Inc., Richmond, VA.
†UNITED KNITTING, Cleveland, TN.
**KAOS Worldwide, Stafford, TX.
††TransTex Technologies International, Plandome, NY.
***KTTEX Corporation, King Tech Group, San Diego, CA.

Since metal additives are prevalently used for performance apparel, both silver- and copper-impregnated fabrics were chosen for this study. Novel silicone-treated fabric was also included for comparison. Table 1 shows the composition of the eight fabrics selected for this study: three polyester (PET) fabrics of different construction and weight doped with copper oxide, three blended fabrics (PET and polyamide [PA]) containing different amounts of silver ions in the nylon fibers, one fabric coated with silver chloride, and one fabric treated with silicone.

Other factors that were not controlled in this study (e.g.,antimicrobial agents mass and availability and fabric construction) could not be examined here.

C. Test Process

Several standard test processes were evaluated, but did not meet study requirements in terms of cost, material quantity, or time. A custom test process was therefore developed for each of the four tests. This test process is described in the following subsection on common test procedures. References are made in the common test procedure to following subsections for each of the four tests.

1. Common Test Procedures

Textiles were cut into 2x2 inch squares and incubated in sterile Petri dishes (Fisher Scientific, Pittsburgh, PA). To ensure that there was limited contact between the textile and the surface of the Petri dish so as to maintain air flow, sterile weighted objects were placed on the bottom of the Petri dish. Textiles in their respective arrangements were then placed on top of weighted objects. Once in configuration, 1mL of a bacterial and fungal consortium consisting of *Staphylococcus epidermidis*, *Escherichia coli*, *Penicillium* species, and *Candida albicans* at concentrations given in the test subsections below was added to each arrangement, but not to the controls. Half a mL of Phosphate Buffer Saline (PBS) was added to sterile the Petri dishes to prevent the textiles from drying.

For this study, a saturation assumption has been made for each microbe concentration in the inoculant. It has been assumed that when each material coupon is inoculated with 1 ml of inoculant, the initial concentration is above the carrying capacity of the coupon due to the material and the area of the coupon. This assumption implies that each coupon is carrying its intrinsic maximum viable concentration at the time of inoculation. Any excess beyond this intrinsic maximum viable concentration either is shed from the coupon or fails to survive.

The importance of the saturation assumption is that a meaningful analysis can be done in terms of colony forming units per milliliter (CFUs/ml) rather than in terms of the proportion of reduction of CFUs/ml from the initial concentration.

The Petri dishes were incubated at 25^{o}C and 98% relative humidity, which is representative of worst-case on-orbit stowage conditions. After an initial incubation period of 16 hours, samples were processed differently, as indicated in the test subsections below.

Following incubation, samples were removed for microbial analyses at the times given in test subsections below. Each textile was placed in a sterile conical tube with 10mL of PBS. After vortexing, serial dilutions were performed for each sample. Aliquots of the serial dilution suspensions were plated on Blood Agar and Sabouraud Dextrose Agar (SDA) for the recovery of bacteria and fungi, respectively. Blood Agar plates were incubated at 35^{0}C for 2 days whereas SDA plates were incubated at 30^{0}C for 5 days. For each sample removal day, there were three treated samples and one control sample per fabric or layer type. Heterotrophic plate counts were completed after incubation and recorded as Colony Forming Units (CFU)/mL.

2. Evaluation of Extravehicular Mobility Unit and Advanced Crew Escape Suit Materials in the Stacked and the Individual Arrangements (Tests 1 and 2)

The ACES and EMU suits are each composed of three garments, as listed below:

- EMU Stack
  - TCU – Capilene® 1 (Patagonia, Inc., Ventura, CA)
  - LCVG (both layers) – nylon tricot and nylon with spandex
  - Bladder – urethane-coated nylon
- ACES Stack
  - TCU – Capilene® 1
  - LCG – Capilene® 4
  - Bladder – GORE-TEX™

Once in configuration, 1 mL of a bacterial and fungal consortium consisting of *Staphylococcus epidermidis*, *Escherichia coli*, *Penicillium* species, and *Candida albicans* at $2x10^3$, 1.4×10^4, 4×10^2, and 2.6×10^5 CFU/mL, respectively, was added to each configuration, but not to the controls.

After an initial incubation period of 16 hours, samples were carefully separated into three individual layers to simulate garments being doffed and stored after an EVA. For the stacked arrangement, each single layer textile was transferred into sterile Petri dishes and returned to the incubator.

Samples were removed from incubation on days 1, 3, 7, 10, and 14 for microbial analyses

3. Evaluation of the Disinfectants on Bladder Materials for the Extravehicular Mobility Unit and Advanced Crew Escape Suit (Test 3)

The primary objective of this test was to identify the efficacy of disinfectants in removing microbes on bladder material for each suit (i.e., the EMU and the ACES) that was evaluated.

Except for controls, 1 mL of a bacterial and fungal consortium consisting of *Staphylococcus epidermidis, E. coli*, *Penicillium* species, and *Candida albicans* at 5×10^3, 1×10^4, 1.7×10^6, and 1×10^3 CFU/mL, respectively, was added to each arrangement

After initial incubation, each textile was wiped with a 2.5×2.5-cm (1×1-in.) foam wipe impregnated with either 10% Stericide [Stepan BTC 2125M], Opti-cide-3® [Micro-Scientific Industries], 50% Maquat® [Mason Chemical Company, Arlington Heights, IL], or 70% isopropanol. Samples were returned to the incubator after cleaning. Samples were removed from incubation on days 1, 3, 7, 10, and 14 for microbial analyses.

4. Evaluation of Antimicrobial Materials (Test 4)

We evaluated eight textiles that were treated with either copper, silver, silver salt, or organic components at various concentrations to ascertain their resistance to microbial colonization.

Except for controls, 1 mL of a bacterial and fungal consortium consisting of *Staphylococcus epidermidis, E. coli*, *Penicillium* species, and *Candida albicans* at 1×10^3, 4×10^3, 2.8×10^5, and $6\text{x}10^3$ CFU/mL, respectively, was added to each arrangement.

Samples were removed from incubation at hours 3 and 7, and at days 1, 7, and 14 for microbial analysis.

D. Randomization

It was necessary to randomize the assignment of coupons to Petri dishes to minimize or to compensate for the effect of any systematic heterogeneous condition or contamination of fabric stock during the course of manufacturing, shipping, and handling. Since the Petri dish labels correspond to treatments to be applied to the coupons, this random assignment is equivalent to a random assignment of coupons to treatments.

The fabric stock was already cut into coupons, and the coupons were already possibly shuffled in the process. This means that the systematic location of the coupons across the stock was lost. However, this still did not assure that the coupons were in a random order with respect to their original location in the stock. This situation was remedied by a random association of coupons with Petri dishes.

For the stacked suit materials, the experiment had progressed too far for randomization to be implemented.

For the individual-layer suit materials experiment, the stack of coupons was taken in its existing order. Individual coupons were assigned to labeled Petri dishes in random order, separately for each coupon type (TCU, LCG, LCVG-L [LCVG-inner layer], LCVGO [LCVG-outer layer], BladderG [ACES GORE-TEX™ bladder], and BladderU [EMU polyurethane-coated nylon bladder]).

The stack of coupons was taken in its existing order for the suit bladder materials experiment. Individual coupons were assigned in random order to the labeled Petri dishes. Additionally, to minimize any systematic effects due to technique in cleansing the bladder coupons with the disinfectant wipes, the order in which the coupons were wiped was also randomized.

The stack of coupons was taken in its existing order for the antimicrobial materials experiment. Individual coupons were assigned in random order to the labeled Petri dishes, separately for each coupon type (Cupron™ NASA-2, Cupron™ NASA-4, Cupron™ NASA-5, UNITED KNITTING 65961, UNITED KNITTING 80031, KAOS, HYGY™, and SILVERCLEAR®).

III. Test Results and Discussion

This section presents the results and a discussion of those results for the following four suit material, disinfectant, and antimicrobial material test:

- Evaluation of EMU and ACES Materials in Stacked Arrangement
- Evaluation of EMU and ACES Materials in Individual Arrangement
- Evaluation of Disinfectants on Bladder Materials for EMU and ACES
- Evaluation of Antimicrobial Materials

The figures in this section are separated into bacteria (*E. coli*) and fungi (*Penicilium* and *Candida*) categories in an attempt to make comparisons easier with current CxP CSSE recommendations. Note that *Staphylococcus epidermidis* was included in the test samples but does not appear in any of the resulting data. This is because all *Staphylococcus epidermidis* CFUs decreased to a statistical zero prior to any sample testing. This may mean that the samples tested are not conducive to *Staphylococcus epidermidis* growth or this biocontaminant was easily overtaken by competing bacteria or fungi. Further testing is required to understand better the absence of *Staphylococcus epidermidis* in all test results.

Since this study measured actual contamination in CFU/mL instead of CFU/cm^2, which is a reference point, defined in CxP CSSE requirements, the following correlations were made: 4×10^6 CFU/100 cm^2 for bacteria is equivalent to 1×10^6 CFU/mL, and 100 CFU/100 cm^2 for fungi is equivalent to 2.5×10 CFU/mL. As a result, each figure in this section containing bacterial data has a dashed line at 1×10^6 CFU/mL, and each fungi-related figure has a dashed line at 2.5×10 CFU/mL. CFU levels below these dashed lines were assumed to be desirable and generally safe to humans. Future discussion and testing may be needed to validate these assumptions. Furthermore, a method for accurately verifying actual bacterial and fungal content in space suit materials may also be essential.

Note that time "zero" in each of the following subsections started when the sample was inoculated, indicating the inoculant concentration. The following subsections present the statistically analyzed data, with error bars denoting a 95% confidence level.

A. Test 1 – Evaluation of Extravehicular Mobility Unit and Advanced Crew Escape Suit Materials in Stacked Arrangement

Bacterial colonization growth in both EMU and ACES stacked arrangement samples (with the exception of the EMU bladder) appeared to increase during the first 3 days, as shown in Fig. 1 and Fig. 3. After the third day, the bacterial levels appeared to reach a somewhat constant state.

The total number of fungal CFUs in both EMU and ACES stacked arrangement samples appeared to slightly decrease with time, as shown in Fig. 2 and Fig. 4. The fungal levels of the TCU, LCG, and LCVG samples appeared quickly to reach a somewhat constant state, whereas the ACES bladder sample showed slight growth between days 1 and 3, followed by a gradual decrease for the remainder of the 14-day test period. All ACES and EMU layers (except the EMU bladder) appeared to exceed CxP CSSE recommended limits for maximum fungal CFU levels.

Bacteria and fungi EMU bladder data for the stacked arrangement also exhibited a significant initial decrease. However, when the statistical data were processed for the EMU bladder case, the number of CFUs was so small the statistical analysis considered these data points to be zero; they were therefore not included in this data analysis.

1. Extravehicular Mobility Unit Stacked Arrangement

Figure 1 shows that bacterial CFU levels in the EMU stacked arrangement TCU and LCVG generally exceed the current recommended CxP CSSE levels for most of the test after the first day. The EMU stacked arrangement polyurethane-coated nylon bladder had insufficient data to be statistically relevant for inclusion in this graph. However, the raw data indicate that CFU levels in the EMU layer appear to be below the recommended CxP CSSE maximum CFU levels for the entire 14-day duration of the test.

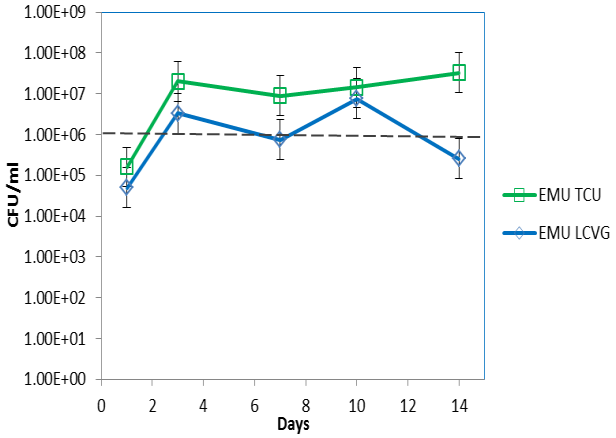

Figure 1. Bacteria heterotrophic plate count (HPC) (CFU/mL) of EMU material in stacked arrangement.

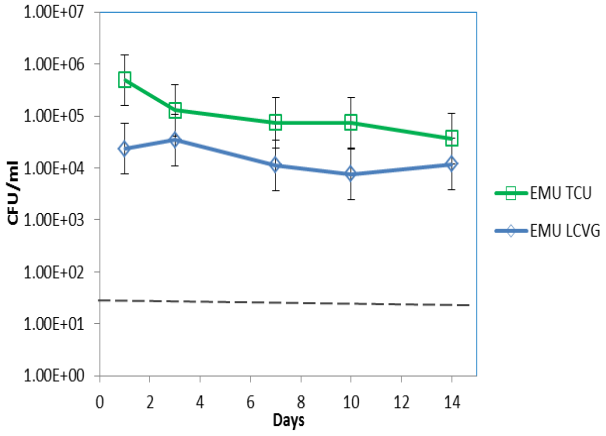

Figure 2. Fungi HPC (CFU/mL) of EMU material in stacked arrangement.

Figure 2 shows that the fungal CFU levels in the EMU stacked arrangement TCU and LCVG generally exceeded the current recommended CxP CSSE maximum levels for most of the 14-day test period. The EMU stacked arrangement polyurethane-coated nylon bladder layer data do not appear on this graph because statistically there was no CFU growth during any of the samples.

2. Advanced Crew Escape Suit Stacked Arrangement

Figure 3 shows that bacterial CFU levels in all layers of the ACES stacked arrangement generally exceed the current recommended CxP CSSE maximum levels for most of the test after the first day.

Figure 4 shows that fungal CFU levels in all layers of the ACES stacked arrangement generally exceeded the current recommended CxP CSSE maximum levels for most of the 14-day test period. The ACES TCU and LCG appeared to follow a similar trend, while the ACES bladder appeared to show a continued decrease in bacterial CFUs through day 14. Additional testing may need to be performed to determine whether this decrease continues to levels recommended by CxP CSSE requirements after 14 days.

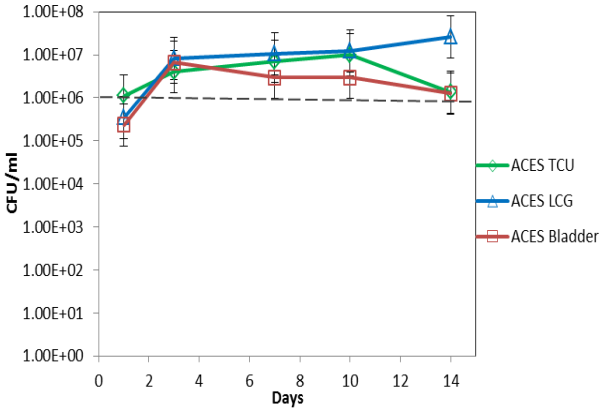

Figure 3. Bacteria HPC (CFU/mL) of ACES material in stacked arrangement.

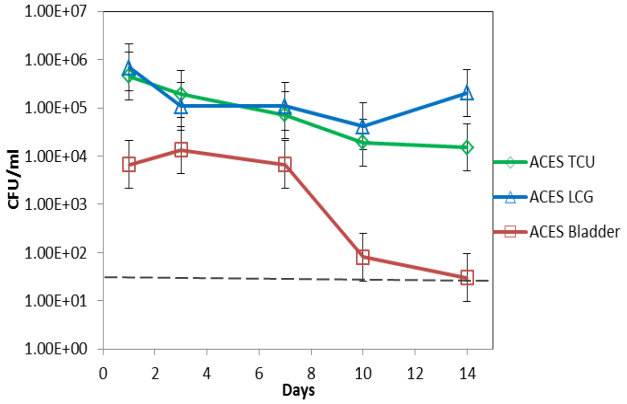

Figure 4. Fungi HPC (CFU/mL) of ACES material in stacked arrangement.

B. Test 2 – Current Suit Material Individual Arrangement

Bacterial colonization growth in the TCU and LCVG-L layers of the EMU individual arrangement samples and all layers of the ACES individual arrangement samples appeared to increase during the first 3 days, as shown in Fig. 5 and Fig. 7. After the third day, these bacterial levels appeared to reach a constant state. This bacterial CFU behavior in the individual TCU and LCVG-L layer arrangements appears to be similar to the behavior found in the stacked TCU, LCVG/LCG, and ACES bladder layer arrangements.

Bacterial colonization growth in the LCVG-O and bladder layers of the EMU experienced an initial decrease during the first day before increasing for the remainder of the 14-day study. Additional testing is needed to determine the reason for the initial decrease in these layers and how many additional days are needed before the bacterial CFUs reach a constant level of CFU/mL.

Fungal colonization growth in all layers of the EMU and ACES individual arrangements showed a noticeable decrease in the first day sample, as shown in Fig. 6 and Fig. 8. The fungal levels in all samples continued to increase after the first day, and did not appear to level off during the 14-day test. Additional testing is needed to determine how many days these fungal CFU increases will continue before they level off or reach a steady state.

Note that since Capilene® is the same material used for both the EMU and the ACES TCU, it was tested once, and the same TCU data were then duplicated in individual arrangement EMU and ACES figures in this subsection for comparison.

1. Extravehicular Mobility Unit Individual Layer Arrangement

Figure 5 shows that, after the first day, bacterial CFU levels in the EMU TCU and LCVG layer generally exceeded the current recommended CxP CSSE maximum CFU levels for most of the test. The EMU LCVG-O and bladder layers showed an initial decrease, followed by continued growth. These layers generally did not appear to exceed the CxP CSSE recommendation for bacterial CFU levels within the 14-day test period.

Figure 6 shows that the fungal CFU levels in all layers of the EMU individual arrangement generally exceeded the current CxP CSSE recommended maximum levels for the duration of the 14-day test period, with the exception of the EMU bladder on day 3. The EMU LCVG-O and bladder fungal CFU levels were noticeably less than those of the TCU and LCVG-L, which is similar to what was observed in Fig. 5 for the bacterial testing. Fungal CFU levels in all EMU layers appeared to continue to increase after the first day for the duration of the 14-day test. Longer testing is needed to determine if these fungal CFU increases level off or reach a steady state.

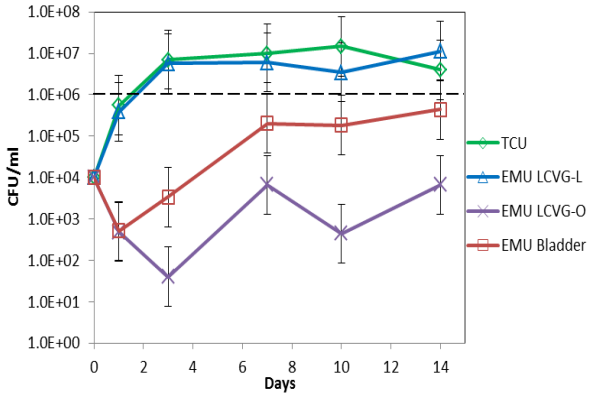

Figure 5. Bacteria HPC (CFU/mL) of EMU material in individual arrangement.

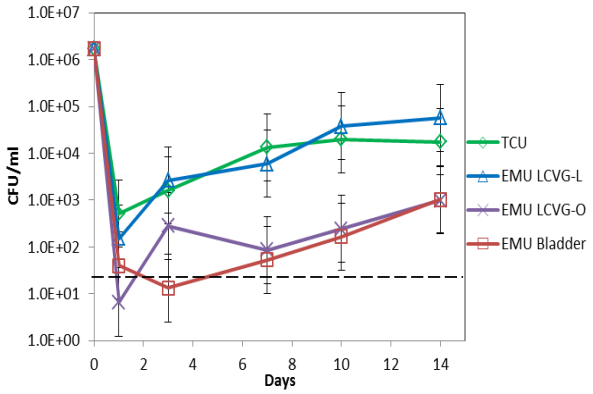

Figure 6. Fungi HPC (CFU/mL) of EMU material in individual arrangement.

2. Advanced Crew Escape Suit Individual Layer Arrangement

Figure 7 shows that bacterial CFU levels in all ACES individual layers generally exceeded the current CxP CSSE recommended maximum CFU levels for most of the test. The behavior of bacterial CFUs in the ACES individual layer appeared to be similar to that of the ACES stacked arrangement, as shown in Fig. 3.

Figure 8 shows that the fungal CFU levels in all layers of the ACES individual arrangement undergo an initial decrease during the first day and then gradually increased or leveled off for the remainder of the 14-day study. This was in slight contrast to the fungi behavior found in the ACES stacked arrangement (shown in Fig. 4), which exhibited the same initial decrease, but then continued to decrease during the remainder of the 14-day study. This may imply that fungi growth in the ACES layers behaves differently in the individual and the stacked arrangements. The fungi levels for both the stacked and the individual arrangements generally exceeded the current CxP CSSE recommended maximum levels for most of the 14-day test period.

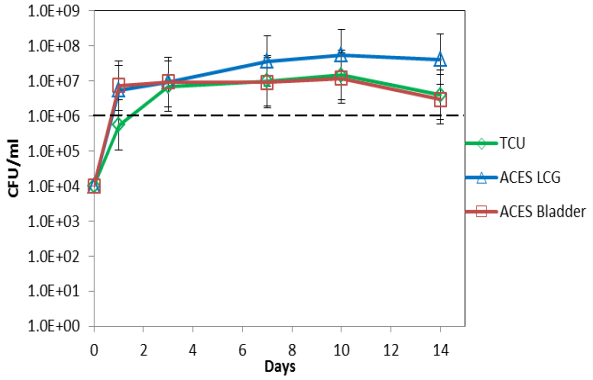

Figure 7. Bacteria HPC (CFU/mL) of ACES material in individual configuration.

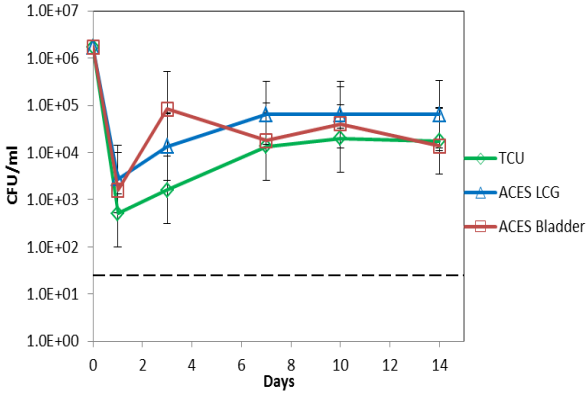

Figure 8. Fungi HPC (CFU/mL) of ACES material in individual configuration.

C. Test 3 – Evaluation of Disinfectants on Extravehicular Mobility Unit and Advanced Crew Escape Suit Bladders

Evidence of bacterial and fungal CFU growth on all EMU bladder samples treated with selected disinfectant was substantially reduced within 7 days, as shown in Fig. 9 and Fig. 10. Evidence of bacterial and fungal CFU growth on ACES bladder material treated with disinfectant is shown in Fig. 11 and Fig. 12. CFU growth on samples treated

with isopropanol or Stericide appears to be similar to individual ACES bladder samples that were not treated with any disinfectant, as shown in Fig. 8 and Fig. 9. Additional testing is needed to determine whether these disinfectants are effective at lowering CFU levels.

1. Disinfectants on Extravehicular Mobility Unit Materials

Figure 9 shows that the bacterial CFU growth for all treated EMU bladder samples was below the current CxP CSSE recommended maximum CFU levels for the duration of the 14-day test. In this figure, EMU bladder samples treated with isopropanol, Opti-Cide-3®, and Maquat® did not have enough data to be statistically relevant due to the large number of samples that contained zero bacterial CFUs 1 day after inoculation. Only the samples treated with Stericide contained measurable amounts of bacteria on days 1 and 3 before going to statistically zero by day 7. Additional testing, including a larger sample set, is needed to facilitate statistically relevant data for these disinfectants. Figure 9 also includes the bacterial CFU growth in the untreated EMU bladder individual layer arrangement, previously shown in Fig. 5. The untreated EMU bladder individual layer is included in this figure to show that each of the disinfectants appears to inhibit bacterial CFU growth better than the untreated bladder alone.

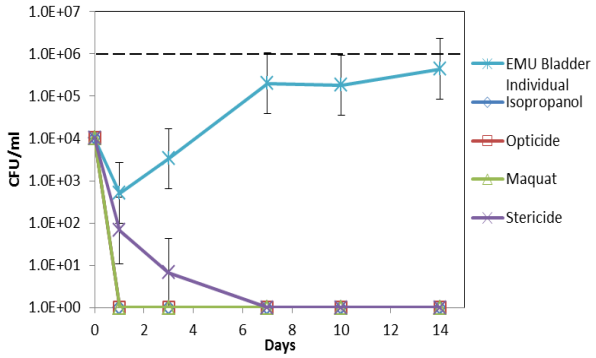

Figure 9. Bacteria HPC (CFU/mL) of disinfected EMU bladder material.

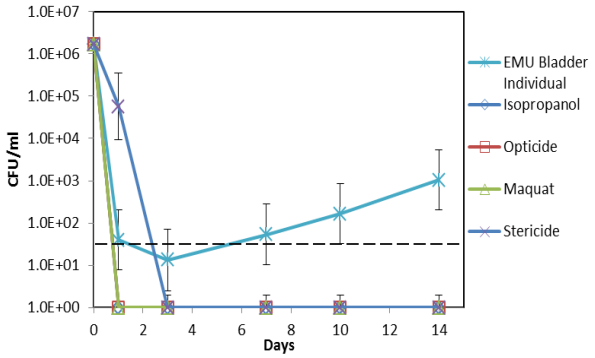

Figure 10. Fungi HPC (CFU/mL) of disinfected EMU bladder material.

Figure 10 shows that fungal CFU growth for EMU bladder treated with disinfectant remained below the current CxP CSSE recommended maximum levels for the14-day duration of this test, with the exception of the first day when materials treated with Stericide exceeded this recommendation. In this figure, the EMU bladder samples treated with isopropanol, Opti-Cide-3®, and Maquat® did not have enough data to be statistically relevant due to the large number of samples that contained zero fungal CFUs 1 day after inoculation. Only samples treated with Stericide contained sufficient amounts of bacteria on day 1 before going to statistically zero by day 7. Additional testing, including a larger sample set, is needed to facilitate statistically relevant data for these disinfectants. Figure 10 also includes the fungal CFU growth in the untreated EMU bladder individual layer arrangement, previously shown in Fig. 5 and Fig. 6. The untreated EMU bladder layer is included in Fig. 10 to show that each of the disinfectants appears to inhibit fungal CFU growth better than the untreated bladder alone, with the exception of the bladder treated with isopropanol on day 1. Additional testing is needed to determine why the bladder treated with isopropanol has higher fungal CFU growth than the untreated EMU bladder at day 1.

2. Disinfectants on Advanced Crew Escape System Materials

Figure 11 shows that bacterial CFU growth in ACES bladder samples treated with Maquat® or Opti-Cide-3® for the ACES bladder generally remained below the current CxP CSSE recommended maximum CFU levels for the 14-day duration of this test. Bacterial CFU growth in ACES bladder samples treated with isopropanol or Stericide remained at higher levels until day 10, when bacteria in these samples began to slightly decrease. Additional testing is needed to determine whether these bacterial CFU levels continue to decrease with time or when they reach a steady state. Figure 11 also includes the bacterial CFU growth in the untreated ACES bladder individual layer arrangement, previously shown in Fig. 7. The untreated ACES bladder layer is included in this figure to demonstrate that each of the disinfectants appears to inhibit bacterial CFU growth better than the untreated bladder alone.

Figure 12 shows that the fungal CFU growth on ACES bladder samples treated with isopropanol or Stericide exceeded the current CxP CSSE recommended maximum CFU levels for the14-day duration of this test. Fungal CFU growth in ACES bladder samples treated with Maquat® or Opti-Cide-3® were statistically zero for days 1, 3, and 7. After day 7, samples that had been treated with Opti-Cide-3® statistically remained at zero while those treated

with Maquat® began to show an increase in CFU growth and exceeded the CxP CSSE recommended levels on day 14. Additional testing is needed to determine whether bacteria CFU levels will continue to increase with time or when they reach a steady state. Figure 12 also includes the fungal CFU growth in the untreated ACES bladder individual layer arrangement, previously depicted in Fig. 8. The untreated ACES bladder layer is included in Fig. 12 to show that each of the disinfectants appears to inhibit fungal CFU growth better than the untreated bladder alone, with the exception of the bladder treated with isopropanol on day 14. Additional testing is needed to determine why the bladder treated with isopropanol has higher fungal CFU growth than the untreated ACES bladder on day 14.

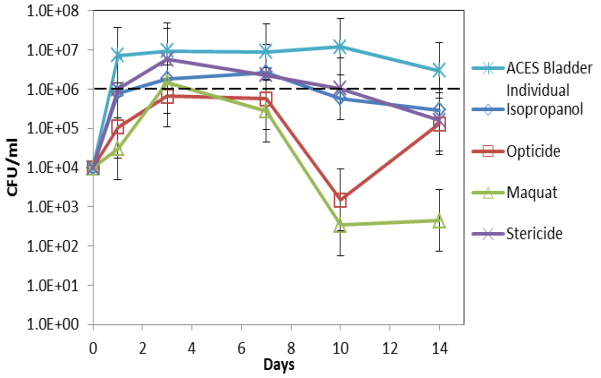

Figure 11. Bacteria HPC (CFU/mL) of disinfected ACES material.

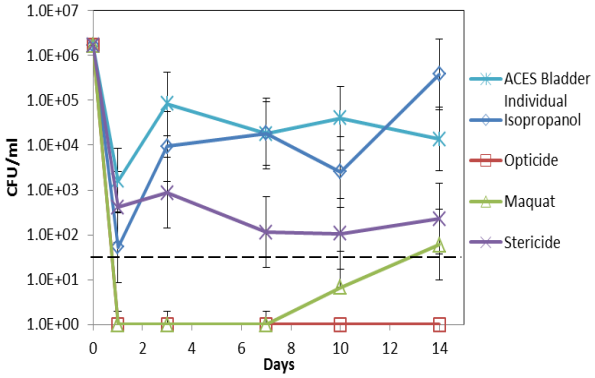

Figure 12. Fungi HPC (CFU/mL) of disinfected ACES material.

D. Test 4 – Antimicrobial Commercial Off-the-shelf Materials

Results for the tested COTS materials were separated into materials that used copper-oxide-doped fiber technology (Fig. 13 and Fig. 14) and those that used either silver-ion-coated fibers or organic treatments (Fig. 15 and Fig. 16). This separation was made to simplify the presentation of the figures in this subsection. Materials with copper-oxide-treated fibers appeared to control bacteria CFU growth better than those with silver-ion treatments. Both ions appeared to have similar behavior related to fungal growth control. Since the copper-oxide fibers were doped and the silver-ion fibers were coated fibers, additional testing needs to be performed to better understand the effect of ions and fiber treatment on CFU growth.

Note that there are no statistical data on samples using SILVERCLEAR® disinfectant because this disinfectant appeared to eliminate all bacteria and fungi within the first 3 hours after application. Statistical statements concerning the effect of SILVERCLEAR® were not possible in this situation.

All copper-doped fiber samples appeared to maintain bacterial CFU levels under the current CxP CSSE maximum CFU recommendation during the 14-day test period. The Cupron™ NASA-2 and Cupron™ NASA-5 samples actually

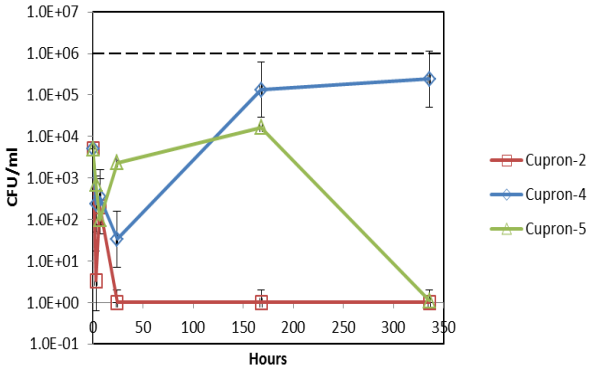

Figure 13. Bacteria HPC (CFU/mL) of copper-doped antimicrobial material.

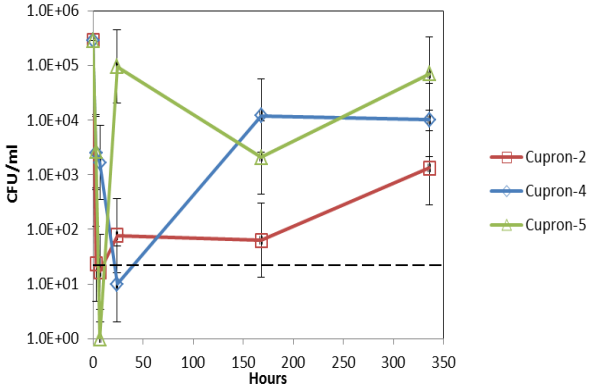

Figure 14. Fungi HPC (CFU/mL) of copper-doped antimicrobial material.

achieved statistically zero bacterial CFU at the end of 14 days, while the Cupron™ NASA-4 sample continued to increase bacterial CFU levels with each sample. Note that all Cupron™ samples used the same type of antimicrobial agent, but they used a different fabric weave. This weave dissimilarity may be responsible for the difference in both bacterial and fungal growth, but further testing is needed to substantiate this observation.

All copper-doped fiber samples appeared to continue to increase fungal CFU levels with time and generally remained above the current CxP CSSE recommended maximum CFU levels for the duration of the test, as shown in Fig. 14.

All silver and organic samples appeared to maintain bacterial CFU levels under the current CxP CSSE maximum CFU recommendation during the 14-day test period; however, HYGY™ and KAOS appeared to be increasing in bacteria CFU levels toward the end of the testing period. If the rates observed between days 7 and 14 continue, these samples may exceed CxP recommended levels, but further testing is required to substantiate this observation.

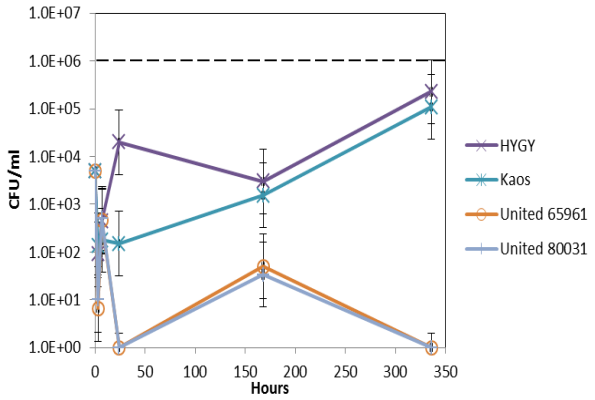

Figure 15. Bacteria HPC (CFU/mL) of silver and organic-based antimicrobial materials.

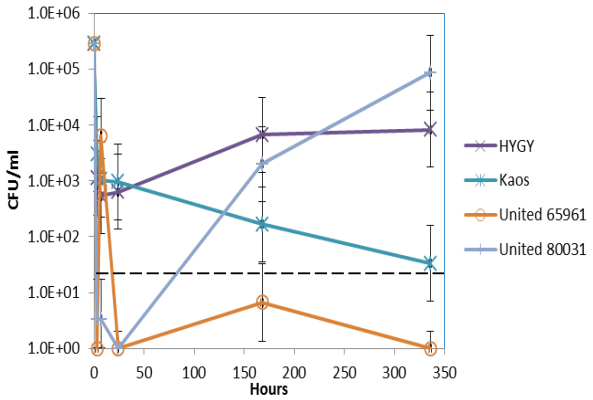

Figure 16. Fungi HPC (CFU/mL) of silver and organic-based antimicrobial materials.

Bacterial and fungal CFU levels for UNITED KNITTING 65961 were statistically zero at the end of the 14-day test period. Fungal levels in HYGY™ and UNITED KNITTING 80031 generally exceeded CxP CSSE recommended levels for maximum CFUs, while UNITED KNITTING 65961 and KAOS showed decreases in CFU levels from day 7 to day 14, as shown in Fig. 16. UNITED KNITTING 65961 appeared to be below recommended CFU levels from day 3 to the end of the 14-day duration test.

E. Statistical Analysis

Negative binomial regression was used to relate the microbial counts to the applied treatments and to calculate the estimated effects and their confidence limits at the follow-up times. For Tests 1, 2, and 3, these follow-up times are 1, 3, 7, 10, and 14 days after inoculation. For Test 4, the follow-up times are 1 and 3 hours and 1, 7, and 14 days after inoculation. The statistical model includes a scale parameter *k*, as follows. If *y* is the number of colony forming units, with mean value μ, then the probability for *y* is

$$p(y)=\frac{\Gamma(y+1/k)}{\Gamma(1/k)\Gamma(y+1)}(k\mu)^{y}(k\mu+1)^{y+1/k},\ y=0,1,2,\ldots.$$

The variance of *y* is $\mu+k\mu^{2}$. The parameter *k* is a scale parameter because the variance of *y* changes linearly with *k*.

In all of the analyses, the mean μ was expanded as a linear function of the effects of treatments, such as microbial type, material type, antimicrobial agent, and incubation time.

The statistical analysis produces no statements about the microbial counts at time zero, since no measurements were taken at that time concerning the effect of the treatments. Statements about increases or decreases in CFUs over time are based on differences in counts from one follow-up time to another and not on differences in counts from the initial microbial concentration. As such, significant differences may imply an increasing or decreasing trend over the follow-up times but not with respect to the initial concentrations. This analysis is based in part on the saturation assumption given above in section II, subsection C, sub-subsection 1, paragraphs 2 and 3.

For the stacked suit materials arrangement, there were no statistically significant differences at the 5% significance level for estimated day-to-day increases or decreases in estimated CFU counts for three out of the four day-to-day differences. Significant increases or decreases at the 5 percent significance level in estimated CFU counts over the entire follow-up period of 14 days are reported in Table 2.

Table 3 summarizes comparisons of significant differences at the 5 percent significance level in estimated CFU counts for selected materials by Microbes and follow-up Day.

Table 2. Overall Follow-up Microbial Change: Stacked Suit Materials

Material	Microbes	p-Value	Significant Overall Increase	Significant Overall Decrease
BladderG	Bacteria	0.0010	Yes	No
BladderG	Fungi	<.0001	No	Yes
LCG	Bacteria	<.0001	Yes	No
LCG	Fungi	0.0134	No	Yes
LCVG	Bacteria	<.0001	Yes	No
LCVG	Fungi	0.3335	No	No
TCU_A	Bacteria	0.0241	Yes	Yes
TCU_A	Fungi	<.0001	No	Yes
TCU_E	Bacteria	<.0001	Yes	No
TCU_E	Fungi	0.0257	No	Yes

Table 3. Selected Sources of Microbial Change Due to Materials: Stacked Suit Materials

Microbes	Day	LCG vs LCVG	TCU_A vs TCU_E
Bacteria	1	Yes	Yes
Bacteria	3	No	Yes
Bacteria	7	Yes	No
Bacteria	10	No	No
Bacteria	14	Yes	Yes
Fungi	1	Yes	No
Fungi	3	No	No
Fungi	7	Yes	No
Fungi	10	Yes	No
Fungi	14	Yes	No

For the individual suit materials arrangement, there were no statistically significant differences at the 5% significance level for estimated day-to-day increases or decreases in estimated CFU counts for three out of the four day-to-day differences. Significant increases or decreases at the 5 percent significance level in estimated CFU counts over the entire follow-up period of 14 days are reported in Table 4. Table 5 summarizes comparisons of significant differences at the 5 percent significance level in estimated CFU counts for selected materials by Microbes and follow-up Day.

Table 4. Overall Follow-up Microbial Change: Separated Suit Materials

Material	Microbes	p-Value	Significant Overall Increase	Significant Overall Decrease
BladderG	Bacteria	0.7979	No	No
BladderG	Fungi	0.0151	Yes	No
BladderU	Bacteria	<.0001	Yes	No
BladderU	Fungi	0.0050	Yes	No
LCG	Bacteria	0.2025	No	No
LCG	Fungi	0.0263	Yes	No
LCVGL	Bacteria	0.0494	Yes	No
LCVGL	Fungi	<.0001	Yes	No
LCVGO	Bacteria	<.0001	Yes	No
LCVGO	Fungi	0.0011	Yes	No
TCU	Bacteria	0.0531	Yes	No
TCU	Fungi	0.0046	Yes	No

Table 5. Sources of Significant Microbial Change Due to Selected Materials: Separated Suit Materials

Microbes	Day	BladderG vs BladderU	LCG vs LCVGL	LCG vs LCVGO	LCVGL vs LCVGO
Bacteria	1	Yes	Yes	Yes	Yes
Bacteria	3	Yes	No	Yes	Yes
Bacteria	7	Yes	No	Yes	Yes
Bacteria	10	Yes	Yes	Yes	Yes
Bacteria	14	No	No	Yes	Yes
Fungi	1	Yes	Yes	Yes	Yes
Fungi	3	Yes	No	Yes	Yes
Fungi	7	Yes	Yes	Yes	Yes
Fungi	10	Yes	No	Yes	Yes
Fungi	14	Yes	No	Yes	Yes

For the disinfectants and suit bladder materials, there were no statistically significant differences at the 5% significance level for estimated day-to-day increases or decreases in estimated CFU counts for three out of the four day-to-day differences. Significant increases or decreases at the 5 percent significance level in estimated CFU counts over the entire follow-up period of 14 days are reported in Table 6. The examination of sources of significant increases or decreases in estimated CFU counts for each follow-up day due to biocidal treatment is summarized in Table 7. Significant increases or decreases in differences of estimated CFU counts due to any of the biocidal treatments (Alcohol, QUAT, or None) are reported in Table 8. Significant increases or decreases in differences of estimated CFU counts due to materials (BladderG or BladderU) are reported in Table 9.

Table 6. Overall Follow-up Microbial Change: Suit Bladder Materials

Biocide	Material	Microbes	p-Value	Significant Overall Increase	Significant Overall Decrease
Alcohol	BladderG	Bacteria	0.4674	No	No
Alcohol	BladderG	Fungi	<.0001	Yes	No

Biocide	Material	Microbes	p-Value	Significant Overall Increase	Significant Overall Decrease
Alcohol	BladderU	Bacteria	1.0000	No	No
Alcohol	BladderU	Fungi	1.0000	No	No
None	BladderG	Bacteria	0.9579	No	No
None	BladderG	Fungi	0.2916	No	No
None	BladderU	Bacteria	0.0007	Yes	No
None	BladderU	Fungi	0.1930	No	No
QUAT	BladderG	Bacteria	0.0688	No	No
QUAT	BladderG	Fungi	0.4886	No	No
QUAT	BladderU	Bacteria	0.5759	No	No
QUAT	BladderU	Fungi	1.0000	No	No

Table 7. Sources of Significant Microbial Change Due to Biocide: Suit Bladder Materials

Material	Microbes	Day	Alcohol vs None	Alcohol vs QUAT	QUAT vs None
BladderG	Bacteria	1	No	No	No
BladderG	Bacteria	3	No	No	No
BladderG	Bacteria	7	No	No	No
BladderG	Bacteria	10	Yes	No	No
BladderG	Bacteria	14	No	No	Yes
BladderG	Fungi	1	Yes	No	No
BladderG	Fungi	3	No	No	Yes
BladderG	Fungi	7	No	Yes	Yes
BladderG	Fungi	10	Yes	Yes	Yes
BladderG	Fungi	14	No	Yes	Yes
BladderU	Bacteria	1	No	No	No
BladderU	Bacteria	3	No	No	Yes
BladderU	Bacteria	7	No	No	No
BladderU	Bacteria	10	No	No	No
BladderU	Bacteria	14	No	No	No
BladderU	Fungi	1	No	No	Yes
BladderU	Fungi	3	No	No	No
BladderU	Fungi	7	No	No	No
BladderU	Fungi	10	No	No	No
BladderU	Fungi	14	No	No	No

Table 8. Overall Microbial Change Due to Biocide: Suit Bladder Materials

Material	Microbes	Day	p-Value	Significant Overall Increase
BladderG	Bacteria	1	0.1910	No
BladderG	Bacteria	3	0.4466	No
BladderG	Bacteria	7	0.4593	No
BladderG	Bacteria	10	0.0661	No
BladderG	Bacteria	14	0.0857	No
BladderG	Fungi	1	0.0381	Yes
BladderG	Fungi	3	0.0055	Yes
BladderG	Fungi	7	<.0001	Yes
BladderG	Fungi	10	0.0003	Yes
BladderG	Fungi	14	<.0001	Yes
BladderU	Bacteria	1	0.2482	No
BladderU	Bacteria	3	0.0002	Yes
BladderU	Bacteria	7	0.9999	No
BladderU	Bacteria	10	0.9999	No
BladderU	Bacteria	14	0.9999	No
BladderU	Fungi	1	0.0004	Yes
BladderU	Fungi	3	0.9999	No
BladderU	Fungi	7	0.9999	No
BladderU	Fungi	10	0.9999	No
BladderU	Fungi	14	0.9999	No

Table 9. Sources of Significant Microbial Change Due to Material: Suit Bladder Materials

Biocide	Microbes	Day	p-Value	BladderG vs BladderU
Alcohol	Bacteria	1	0.9914	No
Alcohol	Bacteria	3	0.9912	No
Alcohol	Bacteria	7	0.9911	No
Alcohol	Bacteria	10	0.9916	No
Alcohol	Bacteria	14	0.9917	No
Alcohol	Fungi	1	0.9942	No
Alcohol	Fungi	3	0.9927	No
Alcohol	Fungi	7	0.9925	No
Alcohol	Fungi	10	0.9931	No
Alcohol	Fungi	14	0.9917	No
None	Bacteria	1	<.0001	Yes
None	Bacteria	3	<.0001	Yes
None	Bacteria	7	0.0453	Yes
None	Bacteria	10	0.0274	Yes
None	Bacteria	14	0.3092	No
None	Fungi	1	0.0511	No
None	Fungi	3	<.0001	Yes
None	Fungi	7	0.0023	Yes

Biocide	Microbes	Day	p-Value	BladderG vs BladderU
None	Fungi	10	0.0040	Yes
None	Fungi	14	0.1723	No
QUAT	Bacteria	1	<.0001	Yes
QUAT	Bacteria	3	<.0001	Yes
QUAT	Bacteria	7	0.9912	No
QUAT	Bacteria	10	0.9914	No
QUAT	Bacteria	14	0.9919	No
QUAT	Fungi	1	0.0003	Yes
QUAT	Fungi	3	0.9934	No
QUAT	Fungi	7	0.9939	No
QUAT	Fungi	10	0.9939	No
QUAT	Fungi	14	0.9937	No

For the antimicrobial materials, there were no statistically significant differences at the 5% significance level for estimated day-to-day increases or decreases in estimated CFU counts for three out of the four day-to-day differences. Significant increases or decreases at the 5 percent significance level in estimated CFU counts over the entire follow-up period of 14 days are reported in Table 10. Table 11 summarizes comparisons of significant differences at the 5 percent significance level in estimated CFU counts for materials by Microbes and follow-up Hour.

Table 10. Overall Microbial Change over Follow-up Times: Antimicrobial Materials

Material	Microbes	p-Value	Significant Overall Increase	Significant Overall Decrease
Cupron2	Bacteria	0.0147	Yes	No
Cupron2	Fungi	0.0011	Yes	No
Cupron4	Bacteria	<.0001	Yes	No
Cupron4	Fungi	<.0001	Yes	Yes
Cupron5	Bacteria	0.0003	Yes	No
Cupron5	Fungi	0.0007	Yes	No
HYGY	Bacteria	<.0001	Yes	No
HYGY	Fungi	0.0294	Yes	No
Kaos	Bacteria	<.0001	Yes	No
Kaos	Fungi	0.0007	No	Yes
United65961	Bacteria	0.0091	Yes	Yes
United65961	Fungi	<.0001	Yes	No
United80031	Bacteria	0.0147	Yes	Yes
United80031	Fungi	<.0001	Yes	No

Table 11. Sources of Microbial Change Due to Materials: Antimicrobial Materials

Microbes	Hour	Significant Change
Bacteria	3	Cupron2 vs Cupron4 Cupron2 vs Cupron5 Cupron2 vs HYGY Cupron2 vs Kaos Cupron4 vs United65961 Cupron4 vs United80031 Cupron5 vs United65961 Cupron5 vs United80031 HYGY vs United65961 Kaos vs United65961 Kaos vs United80031
Bacteria	7	No
Bacteria	24	Curpon4 vs Cupron5 Cupron4 vs HYGY Cupron5 vs Kaos HYGY vs Kaos
Bacteria	168	Cupron4 vs HYGY Cupron4 vs Kaos Cupron4 vs United65961 Cupron4 vs United80031 Cupron5 vs Kaos Cupron5 vs United65961 Cupron5 vs United80031 HYGY vs United65961 HYGY vs United80031 Kaos vs United65961 Kaos vs United80031
Bacteria	336	No
Fungi	3	Cupron2 vs Cupron4 Cupron2 vs Cupron5 Cupron2 vs HYGY Cupron2 vs Kaos Cupron4 vs United80031 Cupron5 vs United80031 HYGY vs United80031 Kaos vs United80031
Fungi	7	Cupron2 vs Cupron4 Cupron2 vs HYGY Cupron2 vs Kaos Cupron4 vs United65961 Cupron4 vs United80031 HYGY vs United65961 HYGY vs United80031 Kaos vs United80031 United65961vs United80031
Fungi	24	Cupron2 vs Cupron5 Cupron2 vs Kaos Cupron4 vs Cupron5 Cupron4 vs HYGY Cupron4 vs Kaos Cupron5 vs HYGY Cupron5 vs Kaos
Fungi	168	Cupron2 vs Cupron4 Cupron2 vs Cupron5 Cupron2 vs HYGY Cupron2 vs United65961 Cupron4 vs United80031 Cupron4 vs Kaos Cupron4 vs United65961 Cupron5 vs Kaos Cupron5 vs United65961 HYGY vs Kaos HYGY vs United65961 Kaos vs United65961 Kaos vs United80031 United65961vs United80031
Fungi	336	Cupron2 vs Cupron5 Cupron2 vs Kaos Cupron2 vs United80031 Cupron4 vs Kaos Cupron5 vs Kaos HYGY vs Kaos HYGY vs United80031 Kaos vs United80031

A detailed examination of effect estimates and their confidence intervals shows inconclusive results that are likely due to an insufficient number of follow-up points and an insufficient number of replicates. Experimental results that show significant day-to-day changes would likely require an evenly spaced follow-up point (e.g., every 2 days) and would also likely require a larger number of replicates (e.g., at least five replicates).

For the stacked and individual arrangement evaluations, a similar examination shows that over the course of the 14 days of follow-up, counts of bacterial CFUs generally increased while count of fungal CFUs generally decreased.

IV. Conclusion

This preliminary trade study was conducted to understand the microbial behavior of the current bladder, LCVG, LCG, and TCU materials and also the on-orbit bladder disinfectants. Disinfectants and antimicrobial fabrics were

selected from a COTS survey and tested in this study. The following conclusions apply to the 14 day duration of the study.

In the stacked material arrangement, neither the EMU nor the ACES suit materials meet the CxP requirements. In the individual arrangement, only the EMU LCVG-O and the EMU Bladder meet completely the CxP bacterial level requirements.

The test of disinfectants on the EMU bladder showed that only the bacterial levels were controlled within the CxP requirements

Among the COTS antimicrobial fabrics, SILVERCLEAR® controlled bacterial and fungal levels within the CxP requirements. The remaining seven antimicrobial fabrics controlled only bacterial levels within the CxP requirements.

V. Summary and Forward Work

We need further understanding of contamination by including more factors in the testing, such as fabric construction (woven, knitted, non-woven), fabric thickness, fiber type, and surface finishes, as well as microbial selection, concentration and mix, and additionally antimicrobial treatments and agents, and also various short- and long-term follow-up times.

The next step in better understanding suit biocontamination should include isolating one or more of the many variables shown in this study. The three leading candidates include: fabric construction, fabric treatment, and microbe type. Since two of these variables primarily apply to the TCU, the next phase of testing should focus on the TCU layer. Future testing will eventually be needed to evaluate the bladder and LCGV layers; however, since the TCU appears primarily to contain the highest CFU counts, this garment is a good focus layer for the next phase of testing.

The first part of the next phase of testing should focus on better understanding the effect of differences in fabric construction on microbial growth. The Cupron™ samples tested in the preliminary study used similar materials and treatments, but different fabric constructions, which is assumed to be one reason for microbial growth differences. Therefore, the first part of this test should use different constructions of a similar base material with similar fabric treatments. These fabric constructions should be subjected to microbial contamination and growth to understand this behavior better.

The second part of the next phase of testing should focus on better understanding the effect of fabric treatment on microbial growth. The results of the antimicrobial materials test suggest that the materials treated with silver- and copper-ion fabrics show potential for controlling microbial growth, but each of these materials also used different base materials and contained different fabric constructions. The second part of this test should use several different silver and copper fabric treatments on similar base materials with similar fabric constructions. These various fabric treatments should be subjected to microbial contamination and growth to understand this behavior better.

The third part of the next phase of testing should focus on better understanding the effect of competing microbial growth. The results in Section III of this paper indicated all *Staphylococcus epidermidis* was eliminated prior to the first sampling, and it is not known whether this was due to competition with other bacteria or fungi. Furthermore, it appeared that there may have been some level of microbial competition between the remaining microbes during the 14-day test period. An extended test period would help to understand better when this competition stabilizes. The next phase of proposed testing will therefore first seek to determine better how each microbe colonizes on antimicrobial materials by itself, to understand better how these microbes behave when placed together in a competing environment. By testing the colonization of only one microbe at a time on similar base materials that have similar weaves and fabric treatments, the colonization behavior of each microbe on antimicrobial materials can better be comprehended.

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[3]NASA, *Constellation Program Extravehicular Activity (EVA) Systems Project Office (ESPO) Space Suit Element Requirements Document (ERD)*, CxP 72208, 2009.