HUMAN FORWARD CONTAMINATION ASSESSMENT: JUST HOW LEAKY ARE SPACE SUITS AND WHAT DO THEY LEAK? M. S. Bell[1], A. B. Regberg[2], M. Rucker[2], A. Hood[2], and M. Walker[2]. [1]Jacobs@NASA/Johnson Space Center, Houston, TX 77058, mary.s.bell@nasa.gov, [2] NASA/Johnson Space center, Houston, TX 77058.

Introduction: When we send humans to search for life on other planets, we'll need to know what we brought with us versus what may already be there. To ensure our crewed spacecraft meet planetary protection requirements—and to protect our science from human contamination—we'll need to assess and verify whether micro-organisms may be leaking/venting from our spacesuits. This requires collecting samples under Extravehicular Activity (EVA) conditions.

Detailed, systematic research on forward contamination from unmanned spacecraft has been steadily progressing since the Viking missions, but systematic studies of contamination from space suits have not been conducted in many years. Space suits use different materials than space craft and are not perfectly closed systems. For example, the modern EMU (Extravehicular Mobility Unit) suit used by NASA is designed to leak at rates as high as 100 cc/min. Before humans land on Mars there is a critical need to understand the types and quantities of microbes that could be introduced via space suits. The Human Forward Contamination Assessment team at NASA's Johnson Space Center (JSC) has developed a prototype EVA swab tool [1] designed for use in space to sample cleaned and uncleaned space suits to determine the present day microbial load and eventually the rate of leakage. The ability to assess microbial leakage early in advanced space suit and life support system design cycles will help avoid costly hardware redesign later. The project has found innovative ways to stretch limited research funds, such as repurposing retired Space Shuttle hardware and piggy-backing onto planned International Space Station, NASA Extreme Environment Mission Operations (NEEMO), and Orion ground tests. Although originally intended to help characterize human forward contaminants, additional potential applications for this tool have been identified, such as for collecting and preserving space-exposed materials to support astrobiology experiments.

Test Objectives: The primary objective of EMU testing was to characterize the type of micro-organisms typically found on or near selected suit pressure joints under suit differential pressure conditions. Most human-borne microbes can fit through a 0.5 to 1.0 μm gap. Knowing which joints are more likely to leak will inform hardware design decisions. Knowing which types of micro-organisms may leak from EVA suits provides a basis for subsequent studies to characterize the viability of those organisms under destination conditions, as well as how far they might spread through natural or human-influenced processes. That data, in turn, will inform exploration mission operations and hardware design.

The secondary objective of testing was to evaluate the interface between a fully suited test subject and the EVA swab tool. Bulky EVA suits can restrict movement and limit visibility through the helmet visor. Fully suited testing is important for identifying tool design issues prior to flight. At exploration destinations, such as Mars, suited crew may be required to periodically sample their suits as part of an environmental monitoring protocol.

This report details results of microbial swabs collected from current flight suit configurations worn by crew members assigned to upcoming ISS expedition missions as well as swabs collected from prototype suits intended for use on the Orion spacecraft. This report also describes the protocols enacted to ensure the scientific integrity of the sampling procedure. These tests were intended to characterize the types of contaminants found on flight suits under current, typical handling conditions. No attempt was made to change suit handling procedures, provide additional sterilization, or to limit typical potential contaminant sources.

The EVA Swab Kit: The prototype EVA Swab Kit used in this test consisted of a single swab tool handle and an eight-canister sample caddy (Fig. 1). The tool

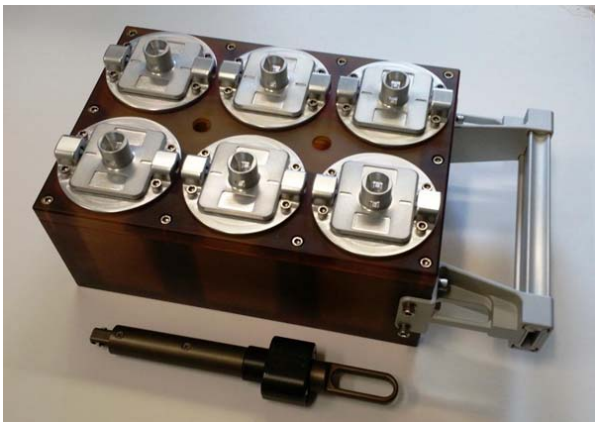

Figure 1. Prototype EVA swab kit.

handle is a heritage Space Shuttle tile repair device. Swab End Effector (EE) Assemblies (Fig. 2) consist of an anodized aluminum holder designed to interface with the tool handle and a paddle-shaped macrofoam

swab tip held in place with two corrosion-resistant steel set screws. Macrofoam swabs are commercially purchased. The swab tips are vendor-certified to be sterile for Deoxyribonucleic Acid (DNA). Each swab end effector assembly is inserted into an anodized 6061 aluminum sample canister assembly. A pair of Delrin® ball detents hold the Swab EE assembly in the canister until use, but allow the swab assembly to be removed with an upward pull on the tool handle. A silicone seal around the top of each EE assembly provides a contamination barrier on one end, while a commercially available, 0.22 micrometer pore microbial filter assembly allows the canister to match ambient pressure without contaminants rushing into the container during atmospheric pressure changes. The filter assembly includes a Delrin Shim, an unlaminated polytetrafluroide ethylene (PTFE) filter and 304 stainless steel perforated disk filter supports.

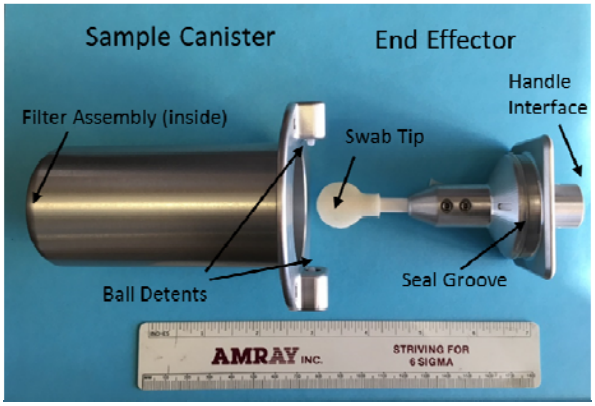

Figure 2. Prototype EVA Swab End Effector & canister Assembly.

The sample canister and EE assembly parts are precision cleaned in the Astromaterials Acquisition and Curation facility utilizing cleaning protocols developed and tested specifically for the materials and configuration of the assemblies (Astromaterials Acquisition and Curation Sample Processing Procedure 177). The cleaned parts are wiped with DNA Away™ and assembled in a laminar flow bench before autocalve sterilization. The sterile parts are then fully assembled in a biosafety cabinet at which time the swab is attached to the EE. The caddy is wiped with IPA (isopropyl alcohol) and the cannisters are then installed to the test-ready configuration in Figure 1.

Test Setup: The tests took place in JSC's SSATA (Space Station Airlock Test Article) which is a human-rated, hi-fidelity, 1-g chamber that provides flight-like simulation of the Airlock and EVA operations in pressures ranging from vacuum to 1 atmosphere. Additional tests were conducted in the 11 ft. chamber, which was able to accommodate 4 test subjects at a time. The chambers were swab sampled in five locations before and after the suited crew sampling for comparison to results of the suit samples. Metallic rings join and seal the glove assembly to the arm assembly of the suits at the wrist; a fabric gauntlet provides thermal protection of this joint. These joints are known to be leak sources. For these test runs, the outer fabric layers (inner and outer gauntlet) and the metallic rings at the wrist joints were sampled. The prototype suits were also sampled along a rear-zipper used to don and doff the suit.

Test Results: At the conclusion of the vacuum test the sample caddy was removed from the vacuum chamber and transferred to a sterile biosafety cabinet. The swabs were removed from the EE and placed into 15 ml of PBS (Phosphate Buffered Saline) in this cabinet. The swabs were then vortexed at maximum power for 5-6 seconds to release the cells from the swab. Individual 0.2 ml. aliquots of PBS were plated onto TSA (Tryptic Soy Agar), BA (Blood Agar) and R2A (Reasoners 2 Agar) for bacterial growth, and PDA (Potato Dextrose Agar) and Saboraud Dextrose Agar for fungal growth. These plates were incubated for 2-7 days and the resulting colonies were counted and identified using a VITEK2[2] instrument or Sanger sequencing. The remaining PBS was used for next generation DNA sequencing. DNA was extracted using a QIAGEN brand bacteremia kit and amplified using PCR (Polymerase Chain Reaction) and the Earth Microbiome Primers for Bacteria, Archaea and Fungi [3]. Amplified DNA was sequenced on an Illumina MiSeq and analyzed using the MOTHUR bioinformatics software[4].

Using culture based techniques, we cultivated 235 CFU (colony forming units) comprised of 26 bacterial species and one fungal species on the outside of the suits. The fungal species and 14 of the bacterial species were unique to the suit surfaces and were not detected in any of the background samples collected within the chambers. 12 of the 14 bacterial species were capable of surviving up to 4 hours at vacuum. The largest number of microbes were collected from the rear zipper area of the suit.

We sequenced 2,464 OTU's (Operational Taxonomic Units, 97% similiarity) from the swab samples. There were 1,489,862 sequences on all of the suit surfaces. 1,199,773 of these sequences represent DNA that survived at least 4 hours at vacuum. The most abundant sequences that survived vacuum belong to the genuses *Staphyloccocus, Ralstona, Bacillus* and *Rhodobacter*. Further analysis of this data is ongoing.

References: [1] Bell, M.S. et al. (2015) LPS XLVI, Abstract #1832. [2] Pincus, D. H. (2005) *Encyclopedia of Rapid Microbiological Methods*. [3] Walters, W. et al. (2016) mSystems 1, 915. [4] Schloss, P. D. *et al.* (2009) *Appl. Environ. Microbiol.* **75,** 7537–41.