

A TOOL TO ASSESS FORWARD CONTAMINATION FROM THE HUMAN MICROBIOME AND PROTECT RETURNED SAMPLES. M.S. Bell¹, R.E. Davis¹, A.B. Regberg², M.A. Rucker², and S.L. Wallace², ¹Jacobs, NASA Johnson Space Center, Mail Code XI3, Houston, TX 77058, mary.s.bell@nasa.gov, ²NASA 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 [1,2,3] 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, piggy-backing, NASA Extreme Environment Mission Operations (NEEMO), and Orion ground tests. The tool has been selected as a secondary activity for extravehicular activity on the International Space Station. 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 (Fig. 1). 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.

Results: 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. 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.

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 surviv-

ing up to 4 hours at vacuum. The largest number of microbes were collected from the rear zipper area of the suit.

We sequenced 755,434 ribosomal fragments on all of the suit surfaces from swab samples. 557,016 of these sequences represent DNA that survived at least 4 hours at vacuum. These sequences formed 2,464 OTU's (Operational Taxonomic Units, 97% similarity) showing low diversity in the samples. The most abundant sequences that survived vacuum belong to the genera *Staphylococcus*, *Ralstonia*, *Bacillus* and *Rhodobacter* all of which are common to the human microbiome. Further analysis of EVA suit materials with respect to the efficacy of various cleaning protocols and engineered containment solutions is planned to inform suit design for NASA's Artemis Moon to Mars program crew testing.

Future Work: The ISS (International Space Station) is an ideal testbed for systematic studies of contamination from crewed vehicles since it has been continuously occupied for 20 years and exposed to non-terrestrial conditions. We will sample the exterior of the ISS during EVA (Extra Vehicular Activity) using a purpose-built swab tool capable of maintaining sterility while undergoing temperature changes from -151 to +121°C under hard vacuum. We will return these sampling tools to Earth and use next generation DNA sequencing to detect, classify, and enumerate microbes. These data will allow us to identify new or improved methods, technologies, and procedures for spacecraft sterilization and leakage mitigation in order to minimize the amount of contamination introduced to the environment by human explorers. Sampling exterior surfaces of ISS will also allow us to characterize the limits of life in the presence of a heat and nutrient source from Earth.

We hypothesize that there is a persistent microbial community on external ISS surfaces. We expect this community to be more robust at locations where nutrient and biomass flux from the interior of ISS is greater (e.g. vents and airlocks). Furthermore, we hypothesize that this community will have genetic adaptations for survival in low pressure, low water activity environments. At the completion of this project we expect to have mapped the ecology of external ISS surfaces us-

ing a tool specifically built for optimal functionality in ISS EVA conditions. This will allow us to make recommendations about how to limit contamination to sensitive destinations like Mars. The tools developed as part of this proposal could be used by robotic missions to collect aseptic samples at vacuum or under harsh conditions like the surface of Europa. Characterizing the microbiology of crewed space craft is also relevant to HEOMD (Human Exploration and Operations Mission Directorate) research goals to study the microbiome of the built environment.



Figure 1. Test subject collecting sample with the EVA swab tool on the wrist joint of the EMU (Extravehicular Mobility Unit) spacesuit currently in use on NASA's ISS (International Space Station). Space suit joints are known sources of leaks but what is leaked and how much leaks are currently unknown.

References: [1] Bell, M.S. et al. (2015) LPS XLVI, Abst. #1832 [2] Rucker et al. (2018) 42nd COSPAR (PPP.3) [3] Bell, M.S. et al. (2019) Mars Extant Life Conference, Abst #5096.