

EVA SWAB KIT: TOOLS AND TECHNIQUES FOR COLLECTING ASEPTIC SAMPLES FROM CREWED SPACE MISSIONS. M.S. Bell<sup>1</sup>, R.Davis<sup>1</sup>; S. Wallace<sup>2</sup>; M. Rucker<sup>2</sup>, A.B. Regberg<sup>2</sup> (<sup>1</sup>mary.s.bell@nasa.gov), <sup>1</sup>Jacobs@NASA/Johnson Space Center), (<sup>2</sup>NASA/Johnson Space Center).

**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 robotic spacecraft has been steadily progressing since the Viking missions, but systematic studies of contamination from space suits has not been conducted in many years. 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 [1,2,3,4] 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.

**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  $\mu\text{m}$  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 at vacuum. 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.

**Suit Microbial Sampling 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. 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. [5] See Danko et al., (2021) for more complete details of these first analyses. 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.

**Swab Tool Function Results:** The kit was demonstrated for fit and function in suited subject vacuum tests to determine how well the tool worked as an aseptic microbial sampling device as well as to identify any design elements that could be upgraded for EVA task specific improvement. It was found that sample acquisition efficacy could be enhanced by redesign of the sample canister to end-effector interface. Several modifications of the sample caddy assemblies to optimize EVA safety and functionality were also identified. Consequently, fabrication of the redesigned sample canister to end-effector assembly interfaces and the sample caddy assemblies are required. Fabrication of sixteen flight sample canister assemblies (8 per each of two EVA Swab Kits) and two sample caddy assemblies are in process to be followed by hardware testing and certification to produce two flight-certified EVA Swab Kits for transport to ISS no earlier than summer of 2022.

**Sampling Strategy:** The 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 using a purpose-built swab tool capable of maintaining sterility while undergoing temperature changes from -151 to +121°C under hard vacuum. Prior to each EVA, the project team will work with ISS mission managers to identify precise sampling locations, which will vary by EVA based on the translation paths and worksites scheduled for that particular EVA. Ideally, translation path handrails and areas near ECLSS (Environmental Control and Life Support System) external vent openings on a spacecraft would be assessed. There are currently more than a dozen ECLSS external vents on the ISS. Some are connected to systems that vent waste products, while others are intended to equalize cabin pressure. As EVA opportunity allows, microbial samples from any of these external vents would provide a valuable data point, though some will be more useful than others. Four criteria have been identified to help prioritize sampling sites near vents:

- EVA Accessibility: *To minimize cost, it is desired to piggy-back onto a planned EVA. Therefore, the sampling location must be readily accessible by an EVA crew*

- Type of Vented Products: *Vent products that have been in direct contact with crew, such as cabin air, are more likely to contain microorganisms than vent products associated with isolated systems, such as experiment module combustion products.*
- Mass of Vented Products: *Higher-flow vents are more likely to contain detectable levels of microbial contaminants than lower-flow vents.*
- Local Environment: *Sample locations with relatively benign local conditions, such as warm surfaces shielded from direct ultraviolet (UV) radiation exposure, may be more likely to support microbial growth than locations with harsher local environmental conditions.*

Because EVA accessibility is the most important criteria, the proposal team worked with an astronaut and flight controllers using the Dynamic Onboard Ubiquitous Graphics (DOUG) tool. The DOUG virtual environment allows an operator to “fly” around the current ISS vehicle configuration to assess EVA translation paths, attach points, and keep-out zones. While analysis on station or rapid return to Earth would be preferable, samples collected from the exterior of the ISS have already been exposed to temperature variations between -157 and +121 °C as well as hard vacuum. Therefore, they should be fairly stable and robust. We hypothesize that samples collected from the ISS exterior could be stored for up to 6 months at -80°C without degradation. Sample canisters will be returned to Earth while frozen at -80°C for analysis, and sterilized canisters can be re-flown back to ISS to support additional sampling opportunities

#### **Relevance to NASA Exploration Objectives:**

These data will allow us to identify new or improved methods, technologies, and procedures for spacecraft sterilization and leakage mitigation to minimize the amount of contamination introduced to the environment by human explorers.

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**References:** [1] Bell, M.S. et al. (2015) LPS XLVI, Abst. #1832 [2] Rucker et al. (2018) 42<sup>nd</sup> COSPAR (PPP.3) [3] Bell, M.S. et al. (2019) Mars Extant Life Conference, Abst. #5096. [4] Bell, M.S. et al., (2020) 43<sup>rd</sup> COSPAR (BO.2). [5] Danko D, et.al., (2021) Front. Microbiol. 12:608478.