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] 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.

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 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% similarity) from the swab samples. There were 755,434 sequences on all of the suit surfaces. 557,016 of these sequences represent DNA that survived at least 4 hours at vacuum. The most abundant sequences that survived vacuum belong to the genera Staphyloccoccus, Ralstonia, Bacillus and Rhodobacter. 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.