ISS External Microorganisms: Tools and Techniques for Collecting Planetary Protection Samples During Extravehicular Activity

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Introduction: We have designed[1], built, and tested a sampling kit (Fig. 1) to aseptically collect microbiological samples from exterior surfaces on the ISS (International Space Station). The kit was flown to ISS as part of the NG-19 commercial cargo mission in August of 2023. Astronauts will use the kit to collect samples from six exterior surfaces on ISS. These samples will be frozen at -80°C after collection and returned to the ground for next generation DNA sequencing. The results of this experiment will help inform planetary protection requirements for crewed missions. We hypothesize that there are detectable microbial communities outside ISS and that these communities originate from inside ISS. Current life support systems do not include any components for reducing microbial leakage. Gases are vented from inside ISS without filtration and there are currently no protocols in place to minimize bioburden on space suit exteriors prior to use. It is important to quantify the bioburden on current vehicles so that achievable limits can be set for crewed missions to astrobiologically relevant locations like Mars.

Figure 1: The sampling kit contains eight swab canisters in total (six on the top and two on the bottom). A resuable end effector (1) is used to remove and reinstall the swabs. The kit also contains a handrail (2), tether loop (3), and, bayonet probes(4) to allow easy manipulation during EVA.

Methods: We have designed a kit to carry 8 commercially available foam swabs into and out of vacuum without compromising the swabs' sterility. The specially designed swab canisters contain a 0.2 µm Teflon filter that allows the canister to accommodate pressure changes without introducing unwanted contaminants. The kit meets existing EVA (Extravehicular Activity) safety requirements and has been used by human test subjects in the neutral buoyance lab and at vacuum in test chambers at the Johnson Space Center. In the early part of 2024, astronauts will use this sampling kit to swab six surfaces on the exterior of ISS. We will collect samples from: the airlock vestibule, the interior surface of the airlock thermal cover, an exterior handrail, a vent connected to the CO_2 removal system, and a vent connected to the payload vacuum system inside ISS. Approximately 300 cm^2 will be swabbed at each location. A seventh swab will be exposed to the vacuum of space without touching any surfaces as a blank. The eighth swab will remain sealed as a process control. After the EVA the swab kit will be frozen at -80°C and returned to earth at the earliest possible opportunity. The samples will remain frozen until they are thawed for next generation DNA sequencing on earth. We will use amplicon sequencing to identify any bacteria, archaea or fungi present in the samples. If there is enough DNA present, we will use shotgun metagenomic sequencing to further characterize the microbial ecology outside ISS.

Preliminary Results: Results from ground-based testing demonstrate that the swab kit is capable of cycling in and out of vacuum without contaminating the swabs. Test subjects, wearing flight-like EVA gloves could remove swabs from the canister and sample discrete locations without inadvertently touching any other surfaces. Several types of bacteria and fungi were collected during these ground tests and survived up to 6 hours at vacuum. This includes non-spore forming bacterial like *Staphyloccus capitis* that are not traditionally considered extremophiles. Shotgun metagenomic sequencing of the ground-test samples revealed bacteria associated with human skin and airways on the exterior of space suits used during these tests [2] In addition to these results we will present preliminary results from our space-flight samples. We will also present lessons learned from attempting to collect microbiological samples during an EVA and describe how our results will affect planetary protection requirements for future crewed missions.

References: 1. Rucker, *et al.*(IEEE, 2018). doi:10.1109/AERO.2018.8396381. 2. Danko, . *et al. Front. Microbiol.* **12**, 1900 (2021). doi: 10.3389/fmicb.2021.608478