

## **ISS External Microorganisms: A Planetary Protection Experiment to Inform Requirements for Crewed Missions to Mars**

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We have developed, tested, and flown a caddy capable of collecting aseptic samples from external surfaces of the ISS (International Space Station). The sampling caddy is certified for use during US EVA (extra vehicular activity) and was launched to ISS in the summer of 2023. We are scheduled to collect samples from 6 locations outside ISS during an EVA in May of 2024. We will freeze these samples at -80°C on orbit and return them to Earth. We will then extract and sequence any DNA collected during the EVA using next generation sequencing technologies to characterize the community composition and function of each sample. Measuring the type and quantity of microbes present on the exterior of ISS will allow us to address knowledge gap 2B, “Acceptable levels of microbial/organic releases from humans and support systems” described in a 2019 COSPAR report<sup>1</sup>. Collecting data about microbial release from current crewed vehicles will inform requirements for acceptable leak rates for future crewed missions to Mars.

The sampling kit consists of eight commercially available, sterile, DNA free, macrofoam swabs ( 23 mm. diameter ) installed in custom aluminum end effectors. Each end effector is housed in an individual aluminum canister. Each canister contains a 0.2 µm Teflon filter to allow the interior volume to accommodate pressure changes without permitting microbial contaminants to enter the sterile interior volume. A handle repurposed from the space shuttle tile repair kit is used to remove the end effector from the sample canister, collect a sample by swabbing a surface and then replace the end effector in its canister. The canisters and end effectors were cleaned and assembled on Earth. Prior to installing the sterile swab the canisters and end effectors were sterilized in an autoclave at 134°C, 215 kPA, for 7 min.. The final assembly occurred in a sterilized class II biosafety cabinet. We will collect six samples from the 1) airlock vestibule, 2) airlock thermal cover, 3) a gap in the micrometeorite shielding near the airlock, 4) a handrail near the airlock, 5) the CDRA (Carbon Dioxide Removal Assembly) vent, and 6) the VES (Vacuum Exhaust System) vent. The remaining two swabs will be reserved as controls. One swab will be exposed during the EVA without touching any surfaces to act as a blank. The final swab will remain sealed until the entire sampling kit is returned to earth. Based on previously published results from the Russian segment<sup>2</sup>, we hypothesize that there will be detectable microbes at some or all of these locations.

Ground-based testing of this sampling caddy confirms that the swabs remain sterile as the canisters transition in and out of vacuum. We were able to retrieve, viable bacterial and fungal cells as well as DNA from samples collected from US space suits during vacuum chamber tests lasting as long as seven hours<sup>3,4</sup>. Based on these results and feedback from the test subjects the sampling caddy was modified to improve ergonomics and meet US EVA safety requirements. Bayonet probes were added to the sides of the sample kit as alternate mounting points. Additional locking features were added to the end effector and the filter stack to prevent inadvertent release during use. The opening mechanism was changed from one where the end effector was rocked laterally to defeat a ball detent to a twist-to-open threaded closure for similar reasons. Demonstrating, this sampling caddy's effectiveness during a US EVA will allow us to address knowledge gaps identified in COSPAR reports and begin to define planetary protection requirements for life support systems on crewed missions to mars. This kit could also be used to collect contamination control samples during Artemis missions to verify requirements and could be easily modified for robotic sample collection.

#### Works Cited

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