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PRE-FLIGHT PREPARATION, SPACEFLIGHT AND GROUND OPERATIONS, AND POST-FLIGHT
PROCESSING OF THE SPACE BIOFILMS BACTERIAL SAMPLES**Abstract**

Biofilms are a problem on Earth given their ability to degrade the materials upon which they grow and due to their relevance to infection processes. For example, 65% and 80% of infections and chronic diseases on Earth are associated with biofilms, respectively. In the case of spacecraft and space stations, these problems are of higher potential impact given that the crew's lives and mission success depend on the nominal operation of mechanical systems. Furthermore, the isolated confined environment (ICE) nature of spaceflight may increase the rates of disease transmission among the crew. In the case of the International Space Station (ISS), biofilms are an identified problem to the Environmental Control and Life Support System (ECLSS), namely on the water processor assembly (WPA). In late 2019, the Space Biofilms experiment launched towards ISS with the objectives of (i) characterizing the mass, thickness, morphology, and associated gene expression of biofilms formed in space with respect to matched Earth controls, (ii) interrogating the expression of genes that confer bacteria with resistance to antimicrobials, and (iii) to test novel material approaches as potential biofilm control strategies for future ECLSS components. For this, 288 samples were prepared prior to the launch of the Northrop Grumman CRS-12 mission (NG-12) from NASA's Wallops Flight Facility in Virginia, USA. This manuscript describes how these samples were prepared prior to flight, including cleaning and sterilization of the 1 cm² coupons of six different materials, and preparation of growth media, *Pseudomonas aeruginosa* inoculum, and fixative and preservative to enable a controlled experiment start and termination on orbit. The integration of these components into the spaceflight hardware, BioServe's Fluid Processing Apparatus (FPA) packed in sets of eight in Group Activation Packs (GAP) is also described. The operational approach, including crew activities (performed on orbit by NASA astronauts Jessica Meir and Christina Koch), use of assets on board ISS, temperature profile, and experiment timeline are described in detail. After fixation/preservation on orbit, the bacterial samples of Space Biofilms returned on board SpaceX' Dragon spacecraft (CRS-19 mission), in early 2020. We here describe how the samples were processed post-flight for data acquisition, including coupon recovery from the spaceflight hardware, biofilm staining process, and storage for potential sample-sharing with other laboratories.

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