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**A lab-on-a-chip platform for bacterial enrichment and single-step RNA purification for rapid screening of spacecraft microbiome (P-004 Lab on Chip)**

**Louisiana Board Of Regents**

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Our approach for rapid detection and monitoring of microbial growth in spacecraft environments is focused on the mitigation of adverse pathogen contamination for both crewed Mars missions as well as robotic exploration. We have developed a Gene Sampling Tool for rapid, dry (without liquid), purification of nucleic acid for subsequent genotyping in microgravity. At the core of the gene sampler technology is a gold-plated stainless steel microscopic pin (130 $\mu$ m $\times$ 3mm) functionalized with synthetic RNA capture sequences for selective purification of bacterial nucleic acid after at least 2 minutes of incubation in the biological specimen. The tool is designed to interface with the Cepheid cycles in orbit and the feasibility of the RNA extraction approach was successfully validated on the International Space Station (ISS) in February 2021 using a radish plant harvested from the APH-02. Building upon this feasibility study results we propose to integrate the Gene Sampling tool with a lab-on-a-chip platform for the selective, aptamer-mediated enrichment of bacterial species followed by on-chip nucleic acid extraction for subsequent genotyping. The microfluidic chip will elegantly interface with the Gene Sampling Tool for dry and selective purification of RNA for subsequent genotyping of the pathogens. In response to identified microbial and health monitoring gaps by the Committee for Space Research (COSTAR), this proposal aims to develop a tool for bacterial enrichment for rapid, selective, and sensitive detection of pathogens in the spacecraft microbiome. One of the crucial obstacles to both human and robotic space exploration is maintaining and monitoring the cleanliness of the spacecraft and instrumentation surfaces. Microgravity increases the risks of microbial proliferation and outbreaks emphasizing the need for rapid pathogen monitoring that eliminates the need for multistep bacterial plating and nucleic acid purification protocols.

Our method offers a one-step nucleic acid purification process that provides faster results and requires less crew time and reduced workspace area. One year of funding is requested to develop a portable platform that does not require large, equipment and eliminates the multistep protocol for additional pre-concentration steps of the bacterial specimen while enabling selective isolation of target bacteria species and genetic analysis. RNA purification and 16S-specific enrichment will be performed using the gene sampler tool for subsequent PCR genotyping experiments. The results of this study will also inform future efforts to integrate the platform with on-chip amplification and gene expression analysis.