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**ORGANIC BIOMARKER-BASED ASSAYS TO EVALUATE TOTAL BIOBURDEN AND ORGANIC COMPOUNDS ON SPACE FLIGHT HARDWARE**

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Meeting planetary protection (PP) requirements for space flight hardware may involve bioburden reduction by dry heat microbial reduction (DHMR) [1]. The NASA standard assay to demonstrate the reduction of organisms involves the swabbing of surfaces, heat shock of the extracted samples, plating of the samples on Trypticase Soy Agar (TSA), and counting colony forming units after an incubation period [2]. The standard assay uses enumeration of heat tolerant spore-formers as a proxy for total bioburden and is generally expected to provide a lower limit. We suggest that a better estimate of the total bioburden could be obtained through sampling and analysis of organic biomarkers. As biological organisms are fundamentally organic in chemistry (i.e. carbon containing materials) it is important to characterize the biomarker compounds that are released from organisms that 1) exist on flight hardware before microbial reduction and 2) left behind from the killed organisms following microbial reduction.

Characterizing biomarkers that remain following microbial reduction is especially important when considering hardware for robotic extraterrestrial missions to detect current or past life on planetary bodies where potentially only biomarkers of organisms remain. The presence of biomarker remains on the spacecraft or sampling hardware of the spacecraft could interfere with the detection of life through biomarker analysis by onboard analytical instrumentation. We envision organic biomarker-based assays that can rapidly detect and quantify the type of biomarker compounds present on flight hardware both before and after microbial reduction.

The experimental evaluation of various organic biomarker assays is important to 1) determine an upper limit of bioburden and 2) insure that space flight hardware for life detection missions are free of organic biomarkers. We propose analogue experiments with coupons doped

with organisms to test organic biomarker compounds both before and after DHMR treatment. Rapid analysis of surface biomarkers will be accomplished by direct analysis techniques, such as direct analysis in real time (DART) and desorption electrospray ionization (DESI) sources coupled with a mass spectrometer. These techniques require no sample preparation and the measurements can be made at the facility where flight hardware is being assembled. From these data, various biomarker reduction strategies can be evaluated and are likely to include specific solvent washes.

[1] NPR 8020.12D Planetary Protection Provisions for Robotic Extraterrestrial Missions; [2] NASA-HDBK-6022 Handbook for the Microbial Examination of Space Hardware