Planetary Protection Research
Abstracts of Selected Proposals
(NNH17ZDA001N-PPR)

Below are the abstracts of the proposals selected for funding for the PPR17 program. Principal Investigator name, institution, and proposal title are also included. Thirteen compliant proposals were received in response to this opportunity. On September 12, 2018, 5 proposals were selected for funding.

Meg Abraham/The Aerospace Corporation
Laser based cleaning and in-situ verification of bio-loading for compliance to Planetary Protection requirements

LIBS based tool for verification and decontamination of spacecraft.

In some cases, the approved cleaning methods prescribed by the NASA Planetary Protection Office (PPO), to meet the maximum bio-loading requirement on higher Category missions, may be not be possible with current life-detect instruments that carry bio chips. This is because the chips cannot be baked or exposed to chemicals. Moreover, a hardware re-contamination of biological materials requires re-cleaning which may not be practical after systems integration. We will propose the application of multi-pulse modulated laser induced breakdown spectroscopy (LIBS) to destroy biological contamination without substrate damage and to create a LIBS spectral library for spacecraft materials that will enable in situ verification of the de-contamination process during cleaning.

Using The Aerospace Corporations renowned laser and detector capabilities coupled with laser amplitude modulation and multipulse techniques already developed, we have assembled an advanced LIBS system that could be miniaturized at some point in the future. We plan to apply the advanced LIBS system to create a spectral library for spacecraft materials and known bio-contamination. Key goals are characterizing LIBS damage thresholds for likely substrates and developing a spectroscopic process that utilizes multi-color, multi-pulse and time domain delay excitation to achieve the best detection limit for verification of requirements at the same time.

LIBS (also known as EMMA) is a microanalysis tool that operates by analyzing the signature of the laser produced plasma to characterize the elemental composition in metals, rocks (e.g. Mars 2020 Rover) and materials. LIBS relies on florescence spectroscopy with its inherently high detection limits. LIBS is useful because only nano to picograms of material need to be removed with minimal sample preparation. A key byproduct is the formation of the plasma which this proposal will demonstrate as a means to removing biologicals from surfaces. In most LIBS tools, the laser repetition rate is kept low to allow the plasma to quench between laser shots. This increases the Signal to Noise for spectrum gathering. Our proposed approach incorporates time-domain modulation of

the laser repetition rate and amplitude to feed the plasma for biological removal with in-between spurts of lower-repetition rate spectrum gathering. Moreover, we intend to enhance the LIBS technique by further developing our double pulse multi-color exposure that will allow characterization of species that are difficult to ionize. These results are possible because The Aerospace Corp has developed a deep knowledge base in precise focused nanoscale laser ablation techniques that is world class. We have designed a LIBS system with significantly more versatility (multiple wavelengths and relation rates) and potentially much lower background with higher detection limits then that found in the literature. We have demonstrated its functional use on Al, Si and Kapton. We will propose a program to continue to investigate wavelength and repetition rate effects to optimize the system for various materials and biological contaminates that are specific to Planetary Protection

The goal is to demonstrate a cleaning and verification method for Planetary Protection requirements through the following investigation: Test a number of spacecraft materials and native bacteria with resulting spectra compared with that in literature, Begin to establish a spectral library of materials and a process for obtaining the best detection limits, Quantify detection limits, purpose our advanced LIBS system with software/hardware control that couples laser amplitude modulation and multipulse techniques such that they are optimized for Planetary Protection applications.

Lori Fenton/SETI Institute
Assessing the Potential for the Forward Contamination of a Robotic or Human Exploration Zone by Aeolian Processes on Mars

We will address three key science questions relevant to the forward contamination of robotic and human Exploration Zones (EZs) on Mars:

1. How are microorganisms removed from spacecraft surfaces by clear, dust-laden, and sand-laden winds, and what spacecraft materials and configurations best protect against this removal?

2. How quickly will bacterial and fungal spores be inactivated under simulated Martian conditions?

3. How far might viable microorganisms be transported by the wind on Mars?

The first question will be addressed with the NASA Ames Research Center's Mars Wind Tunnel (MARSWIT), which can simulate aspects of wind-driven particle transport at Mars-like air pressures. Various types of coupons doped with fluorescent microspheres (FLMS), coated with simulated extracellular polymeric substances (EPS) to produce adhesion, will act as proxies for spacecraft surfaces contaminated with microorganisms. The coupons will be placed at various positions on a range of mock-up spacecraft components and subjected to aeolian abrasion. MARSWIT will be run at a range of wind speeds and with a range of dust and sand sizes as erosional agents. These experiments

will empirically define the conditions that lead to removal of microbial surrogates, thereby defining the likelihood of forward contamination from landed spacecraft on Mars. Specific experiments will utilize inactivated (dead) spores to verify that the FLMS are mimicking microbial adhesion and wind removal processes in the MARSWIT tests.

To address the second question we will study the survival of bacterial and fungal species not previously tested under simulated Martian conditions. The proposed work will test the survival rates of 4 bacterial and 2 fungal species exposed to Mars-like conditions of solar UV irradiation, air pressure (10 mbar), temperature (-60 to +20 degrees C), and a gas composition consisting of the top 5 gases in the Martian atmosphere. The microbial strains will be selected from isolates recovered from Mars spacecraft surfaces prior to launch. For example, B. safensis (InSight), B. amyloliquefaciens (InSight), and Acinetobacter radioresistens 50v1 (Odyssey) will act as new species tested for survival under Martian conditions. The fungal species Aspergillus niger and Fusarium oxysporum will be included in the assays to include eukarya that have been identified on spacecraft surfaces.

The third question will be addressed with large-eddy simulations (LES) using the Mars Regional Atmospheric Modeling System (MRAMS), a numerical climate model capable of simulating microscale atmospheric phenomena on Mars. Aeolian transport of microorganisms will be represented in the LES as tracers, using spacecraft removal conditions determined from MARSWIT tests to parameterize a point source in the center of the LES domain (e.g., the result from question #1). As they are transported by the wind, microorganism viability will be determined by applying biocidal kill kinetics to the tracers (e.g., the result from question #2). LES will be conducted for three locations on Mars, at four different seasons and two different local times each (i.e., daytime and nighttime) in order to model a broad range of environmental conditions.

Resulting data products from the LES will consist of isopleths of microbial concentrations (i.e., mass per unit area) over time. This work will produce the first bounds on the likelihood and distance of viable microbial transport on Mars, providing a resource for future reassessments of planetary protection policy and for mission planners seeking to avoid false-positives when designing and planning operations for instruments to investigate martian life.

Hernan Lorenzi/J Craig Venter Institute, Inc.
Molecular detection, classification and quantification of spacecraft-associated microbial and eukaryotic organisms and their potential for surviving in extraterrestrial environments.

Since the beginning of space exploration there is a concern about interplanetary cross-contamination. Robotic vehicles and the exteriors of manned spacecraft represent extreme environments for Earth organisms due to the physical extremes of outer space and the thorough decontamination procedures applied to spacecraft before launch.

However, recent studies show that mamy microorganisms are able to withstand these harsh environments/procedures and even survive under Mars-like conditions.

Current NASA standard procedures for assessing microbial bioburden on spacecraft involve culture based methodologies that are time consuming. Also, a serious limitation of this approach is that most microbial organisms are non-cultivable and hence, it is likely to severely underestimate the level of microbial contamination. Here we propose to develop an efficient and sensitive molecular methodology for the rapid identification and quantification of contaminating microbiota based on LAMP amplification and MinION sequencing. To achieve this goal, we propose the following specific aims:

Aim 1: Quantitative and qualitative analysis of microbial species present on the surface of cleanrooms before, during and after spacecraft assembly in preparation for the InSight Mission. We will perform bacterial 16S/fungal ITS1 amplification by qPCR followed by Illumina sequencing of samples collected from cleanroom surfaces at key time-points during mission preparation. Qualitative and quantitative comparison of microbial species profiles before and after PMA treatment will allow us to construct a catalog of organisms able to endure the decontamination treatment applied to spacecraft and cleanroom surfaces.

Aim 2: Genomic characterization of microbial communities that withstand spacecraft decontamination processes and assess their capacity to endure outer space or extraterrestrial environments. We will identify genetic signatures specific of microorganism resistant to decontamination and/or extraterrestrial environments through whole genome sequencing and analysis of 180 bacterial isolates that have been shown to survive in conditions similar to those found on the Martian surface. In addition, we will perform a metagenomic sequencing approach to characterize the metabolic capacity of the microbial communities present in the InSight samples and evaluate their potential of surviving on the Mars surface by comparative analysis against metagenomic samples from relevant extreme environments and whole genome sequencing data from the 180 resistant isolates.

Aim 3: To implement a rapid and cost-effective LAMP 16S/ITS1 amplification and MinION sequencing protocol for the identification of microbial contaminants present on the surface of assembly cleanroom. We will evaluate the performance of MinION technology during the characterization of extant microbial communities from InSigt samples compared to Illumina-based taxonomic profiles from Aim 1. We will also develop a LAMP protocol to amplifying 16S and ITS1 molecular markers that will then be sequenced with MinION. Finally, we will adjust JCVI 16S/ITS1 Illumina processing pipeline to work on MinION reads derived from LAMP amplicons to improve MinION-based taxonomic profiling.

The proposed research is relevant to the Planetary Protection Research Program in that its main goal is to develop a novel, sensitive molecular analytical methods for the rapid identification and quantification of a broad range of microbial organisms to assess the likelihood of forward (and potentially backward) contamination.

Significance: Once developed, the proposed methodology will help setting new, more sensitive and informative standards for assessing bioburden during spacecraft preparation and operating procedures on Earth, that can potentially be applied during space missions or in other planets.

Scot Rafkin/Southwest Research Institute
Modeling the Mobilization and Atmospheric Transport of Biological Material and Contaminants from Humans and Spacecraft on Mars.

A major motivation for the exploration of Mars is the search for extant life, past or present. The identification of indigenous biological material or life on Mars requires reasonably excluding the possibility that the sample in question is of terrestrial origin (i.e., the forward contamination problem). Although planetary protection policies are in place to limit the biological load on spacecraft that enter the Mars system, it is not possible to completely sterilize hardware; every spacecraft that goes to Mars carries some amount of organic and biological material.

Given that biologically contaminated hardware (and eventually people) will continue to go to Mars, it is important to understand under what conditions contaminants can be mobilized and transported. It is also important to understand to where the contaminants might be transported with particular attention to the location of known "special regions". Consequently, the goals of the proposed effort are two-fold:

Goal 1: Mature and demonstrate at three high-priority exploration locations an end-to-end modeling system that predicts the regional transport fate of contaminants.

Goal 2: Identify optimally protective landing site locations that minimize transport contamination of nearby special regions or other places of interest.

The attainment of these goals requires achievement of the following objectives:

Objective 1: Develop a contaminant mobilization parameterization based on existing dust/sand lifting physics and additional physical constraints and considerations.

Objective 2: Utilize existing Mars modeling capabilities and adapt existing terrestrial transport models to Mars to predict the regional dispersion and transport of contaminants.

Objective 3: Employ back-trajectory calculations to identify dominant regional air sources of special regions.

The overall approach is to closely follow the methods used to determine and quantify transport of pollutants and hazards on Earth. On Earth, mesoscale models are coupled to Lagrangian transport models to predict where a release of material will go as a function of time. The same modeling tools can also be used to identify the likely source of

contaminants for a given location. For Mars, mesoscale model tools are widely available while Lagrangian modeling tools for Earth can be adapted for Mars with a modest and well understood effort. For this project the Mars Regional Atmospheric Modeling System (MRAMS) is selected as the mesoscale model and HYSPLIT is selected as the Lagrangian model. At the completion of this project, an atmospheric transport modeling system will be demonstrated at three locations of high-priority interest to the Mars Exploration Program (MEP).

The Planetary Protection Research Program (PPRP) specifically solicits proposals that, "Model planetary environmental conditions and transport processes that could permit mobilization of spacecraft-associated contaminants to locations in which Earth organisms might thrive, for example Mars Special Regions..." The proposed work does exactly this. It addresses the mobilization and transport of contaminants using site-specific modeling, taking into account the environmental conditions that mobilize and transport contaminants, but also factoring in the environmental conditions that lead to the destruction of contaminants as well as predicting the microclimates that might foster propagation.

Contamination potential is site specific. It is not possible to determine the potential at every place for all time. Environment conditions change as a function of time and day and season. Further, the potential source of contamination and mobilization potential is likely to be mission specific. Thus, it is important to develop and demonstrate the tools to individually assess specific exploration landing sites on an as needed basis. This study does that.

Parag Vaishampayan/Jet Propulsion Laboratory
Genome Encyclopedia of Spacecraft Associated Microbes (GESAM).

NASA Planetary Protection engineers have been continually monitoring bioburden on spacecraft and associated surfaces since the Viking I mission using the NASA Standard Spore Assay. Recently, researchers have employed a variety of next generation sequencing technologies and metagenomics approaches to further explore the biosphere present in cleanrooms. However, these culture-independent tools do not allow researchers to parse the metabolically active organisms which pose a more serious threat to Planetary Protection implementation, than the mere presence of extraneous DNA or non-viable organisms. Metagenome analysis, while valuable for many purposes, often provides an ambiguous functional profile due to incompletely assembled genomic data and unavailability of reference genomes of close relatives to organisms found in the sample. An expanded reference genome set of spacecraft surfaces will improve the ability to make useful interpretations from past, present and future culture independent studies (e.g., better functional predictions from rRNA gene surveys, better assembly of metagenomics data). Reference genomes are also critical for meta-proteomic and meta-metabolomic studies because they provide the ability to make catalogs of potential peptides and metabolites. Most importantly, to understand the dominant and reoccurring microbial population from spacecraft surfaces, no samples, DNA or sequence data are available from past missions. Fortunately, the planetary protection group at JPL has

isolated and archived over 5000 bacterial isolates from past NASA missions. Of these isolates, the taxonomic identity of fewer than 1500 isolates and almost no whole genome sequence data are available. This knowledge gap has imposed serious limits on our understanding of the microbial diversity and functional characteristics of these isolates.

The key objectives of the GESAM project are (1) taxonomic identification of all the bacterial isolates (>5000) currently present in the JPL Planetary Protection microbial archive using MALDI-TOF MS coupled with 16S rRNA sequencing approach, (2) Whole Genome Sequencing (WGS) of the most abundant, most re-occurring and potentially novel microbial species (~500), (3) comparative functional genomics and pan-genome analysis using the WGS data.

This project will aim to develop a reference genome set, which will provide new insights into the genetic variability and functional capabilities of microorganisms isolated from spacecraft surfaces. Such knowledge will enhance NASA's ability to gauge the planetary protection risks by understanding the functional attributes relevant to microbial survival in extraterrestrial environments. Microbes which are of particular interest and application to planetary protection are those which are the likeliest to persist harsh cleaning procedures (vapor hydrogen peroxide, dry heat, and cleaning solutions), survive interplanetary transfer (gamma, UV radiation, and desiccation), and proliferate on extraterrestrial planetary bodies (employing a diverse set of metabolic capabilities, anaerobes, and psychrophiles). Therefore, understanding the presence of these unique characteristics in strains directly isolated from spacecraft hardware will allow PP researchers to more accurately assess the likelihood of their interplanetary transfer and survival on extraterrestrial planetary bodies.