Fiscal Year:	FY 2011	**Task Last Updated:**	FY 02/07/2012
PI Name:	Pierson, Duane L Ph.D.		
Project Title:	A Comprehensive Characterization of Microorganisms and Allergens in Spacecraft Environment		
Division Name:	Human Research		
Program/Discipline:	HUMAN RESEARCH		
Program/Discipline--Element/Subdiscipline:	HUMAN RESEARCH--Environmental health		
Joint Agency Name:		**TechPort:**	No
Human Research Program Elements:	(1) **SHFH**:Space Human Factors & Habitability (archival in 2017)		
Human Research Program Risks:	(1) **Microhost**:Risk of Adverse Health Effects Due to Host-Microorganism Interactions		
Space Biology Element:	None		
Space Biology Cross-Element Discipline:	None		
Space Biology Special Category:	None		
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Zip Code:	77058	**Congressional District:**	22
Comments:			
Project Type:	FLIGHT	**Solicitation / Funding Source:**	99-HEDS-03
Start Date:	07/01/2002	**End Date:**	09/30/2011
No. of Post Docs:	0	**No. of PhD Degrees:**	
No. of PhD Candidates:	0	**No. of Master' Degrees:**	
No. of Master's Candidates:	0	**No. of Bachelor's Degrees:**	
No. of Bachelor's Candidates:	0	**Monitoring Center:**	NASA JSC
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Flight Program:	Shuttle/ISS		
Flight Assignment:	ISS STS-115, STS-116, STS-117. STS-118, STS-120, STS-121 NOTE: End date is 9/30/2011, per HRP information (Ed., 10/20/2011) NOTE: End date is now 9/30/2009 per CoI (4/08)		
Key Personnel Changes/Previous PI:			
COI Name (Institution):	Cruz, Patricia (Harry Reid Center for Environmental Studies) Ott, C. Mark (National Aeronautics and Space Administration-JSC)		
Grant/Contract No.:	Internal Project		
Performance Goal No.:			
Performance Goal Text:			

	This study of microorganisms, allergens, and microbial toxins in the spacecraft environment was initiated to ensure the health, safety, and performance of crewmembers during flight. As all previous methods evaluating spacecraft ecology utilized culture-based methodology, this study focuses on techniques that can identify most of the previously omitted microorganisms, such as the pathogen Legionella pneumophila, the etiological agent of Legionnaires' disease. Likewise, culturable bacteria and fungi have been the only potential allergens studied; the more potent allergens, such as dust mites, have never been analyzed in spacecraft environments. No previous study has targeted microbial toxins. This study utilizes modern molecular biology, advanced microscopy, and immunochemical techniques to examine air, surface, and water samples for bacteria and fungi (total composition and specific pathogens), allergens (e.g., dust mites), and microbial toxins (e.g., endotoxin and volatile organic compounds).
Task Description:	This study of the International Space Station (ISS) will include (1) sampling and analysis of ISS modules immediately prior to launch to develop baseline levels of contamination, (2) direct on-orbit sampling of the ISS and subsequent ground analysis. This study will reveal previously undetected microorganisms, allergens, and microbial toxins in the spacecraft environment, which we anticipate will result in a more comprehensive health assessment of spacecraft during extended missions. See also https://
Rationale for HRP Directed Research:	
Research Impact/Earth Benefits:	The results of this study will provide insight into changes that occur in the microbial ecology of semi-closed systems. While this study is designed to predict trends in spacecraft, it can be applied to terrestrial systems such as office buildings and residential homes. The development of specific primers for bacterial enumeration and fungal identification during this study will also advance the ability of ground-based investigators to diagnose the potential sources of microbial contamination and give insight into the causes of "sick building syndrome."
Task Progress:	Throughout the SWAB flight experiment, multiple notable accomplishments were achieved. Sample collection was initiated in August 2006. Air and surface samples, including 9 in-flight sessions and multiple preflight samples, were completed in March 2008. ISS water sample collection from the U. S. water regeneration system called the Water Process Assembly (WPA) began in August 2009 and was completed in March of 2010. The following describes advances in hardware development, the use of molecular technical for spaceflight applications, our understanding of microbial ecology on ISS. Collectively, the SWAB flight experiment provided tremendous benefits toward our approach to infectious disease risk associated with spaceflight. Hardware development. Prior to the SWAB experiment, sample collection focused on subsequent processing for culture based analysis. For sample collection, the paradigm shifted from preservation of culture viability to preservation of the DNA integrity. Potable water and surface samples relied on DNA fixatives to maintain the DNA. Air sample integrity was achieved by using filtration through a gel membrane (Sartorius MD8 Air Port air sampler). This development effort will be beneficial for future experimental and monitoring efforts, as exemplified when the Sartorius air sampler from the SWAB experiment remained on board ISS at the request of JAXA investigators, who intend to use it in completion of their microbial ecology experiment. Within the design process were multiple side experiments to enable this effort. These side experiments will prove useful in future endeavors. Indeed, the simple determination of average extractable DNA from ISS potable water has already been used to size multiple flight experiments and will be used in the development of next-generation flight hardware. Molecular techniques and microbial ecology. The development of molecular techniques for the SWAB flight experiment provided advances in the NASA laboratory processes associated with microbial identification. The first accomplishment of the SWAB experiment was the translation of 16S ribosomal DNA sequencing for the identification of bacteria from flight experiment to operational use on spaceflight samples to determine crew health risk. The use of this molecular technique increased bacterial speciation of environmental isolates three fold compared to conventional biochemical-based methodology. This increased efficiency in bacterial speciation provides a better understanding of the microbial ecology and the potential risk to the crew. Negative aspects concerning the use of molecular techniques for spaceflight applications were also identified during the development phase of the SWAB experiment. Sample preparation, data analysis, and potential contamination by genetic material pose tremendous challenges for future use of molecular identification during future exploration missions. Early analyses for the SWAB experiment focused on the use of molecular-based DNA fingerprinting using repetitive sequence-based polymerase chain reaction (rep-PCR). This technology has allowed contamination tracking of microorganisms between crewmembers and their environment. This study not only demonstrated that ISS has a greater diversity of organisms than originally expected, but also provided insight into possible routes of infection to the crew. Additional ground-based studies used rep-PCR and protein based assays to determine the potential of methicillin resistant Staphylococcus aureus (MRSA) aboard ISS. MRSA has become increasingly common on Earth and poses a treatment problem for infections during flight. The first technique used to evaluate all DNA from the flight samples was Denaturing Gradient Gel Electrophoresis (DGGE). Unlike other techniques, DGGE does not depend on microbial growth on culture media, allowing a more comprehensive assessment of the spacecraft interior. The results indicated the presence of microorganisms not commonly isolated from surface and air samples using culture based techniques. While no medically significant organisms were detected using DGGE, results indicated that DGGE was much less sensitive than culture-based methods. The use of Real Time PCR (RT-PCR) assays was exceptionally beneficial, as this technique proved more sensitive than DGGE. Analyses focused on the sensitive, targeted analysis of DNA for specific viruses including Varicella Zoster Virus (VZV), Cytomegalovirus (CMV), and Epstein Barr Virus (EBV). This technique detected the presence of VZV and EBV DNA in a number of the surface and air samples. An RT-PCR detection assay specifically for the detection of Stachybotrys chartarum and Aspergillus fumigatus was performed on ISS air and surface samples. No ISS samples indicated the presence of either organism. An additional RT-PCR assay investigating the presence of methicillin resistant Staphylococcus aureus was developed using custom designed primers and probes and performed on all of the surface and air DNA samples.

	The search for medically significant organisms using non-culture based technology did not reveal a large number of previously unseen medically significant organisms, thus providing a better understanding of the true microbial ecology that is experienced by the crew during flight. This information is leading toward an accurate microbial risk assessment to help set flight requirements to protect the safety, health, and performance of the crew.
Bibliography Type:	Description: (Last Updated: 03/24/2020)
Articles in Peer-reviewed Journals	Vesper SJ, Wong W, Kuo CM, Pierson DL. "Mold species in dust from the International Space Station identified and quantified by mold-specific quantitative PCR." Res Microbiol. 2008 Jul-Aug;159(6):432-5. https://doi.org/10.1016/j.resmic.2008.06.001 ; PubMed PMID: 18602989 , Jul-2008