Isolation and Monitoring of Cleanroom-Associated Microbial Contaminates From Geological Collections. R. E. Davis[1], C. L. Castro[2], S. L. Castro-Wallace[3], F. M. McCubbin[4], and A. B. Regberg[4], [1]Jacobs, JETS Contract, NASA Johnson Space Center, 2101 NASA Parkway, Houston TX 77058, [2]JES Tech, 16870 Royal Crest, Houston TX 77058, [3]Microbiology, NASA Johnson Space Center, 2101 NASA Parkway, Houston TX, 77058, [4]Astromaterials Research and Exploration Science Division, NASA Johnson Space Center, 2101 NASA Parkway, Houston TX, 77058

Introduction: Microbial contamination is of particular interest to geological curation as many microorganisms can change mineral composition and produce compounds used as biosignatures used for the detection of life[1]. Microbial cells can change the mineral composition of rocks through organic acid production and direct enzymatic oxidation/reduction of transition metals. Enzymatic oxidation of iron and manganese can occur at a rate several orders of magnitude faster than under abiotic conditions and produce highly reactive nanoparticle-sized oxides that can react and sorb other metals and organic compounds[2]. Many fungi can also produce organic acids that dissolve and chelate mineral matrices chemically reducing and dissolving rock surfaces[3]. Finally, several common soil-associated bacteria and fungi produce secondary metabolites that contain unusual amino acid analogs and non-ribosomal peptides containing both L- and D- chirality used in characterizing carbonaceous chondrites and the detection of extraterrestrial life[4].

Methods: We utilized the meteorite and lunar curation labs maintained by the Astromaterials Acquisition and Curation Office at Johnson Space Center. A comprehensive microbial sampling and monitoring plan was implemented in November 2017 for these cleanrooms[5]. Sampling sites were chosen based on surfaces with high potential for contamination and areas of direct contact with critical samples. For solid surfaces, such as floors and tabletops, 300 cm^3 areas were swabbed with sterile polyester- and foam-tipped swabs. These swabs were immediately placed in sterile 50 ml centrifuge tubes to which 15 ml of sterile phosphate buffered saline was added and vortexed to transfer the cells to the solution. This solution was used to inoculate media in Petri dishes for growth as described previously[5]. Microbial isolates were aseptically transferred to new plates and identified using either a VITEK automated system, sequencing the ribosomal small subunit gene, or morphologic features in the case of fungi. Recovery rate was calculated as the percentage of growth plates showing microbial growth in each sampling site or sampling event[6].

Results: The Meteorite Processing Lab had a mean recovery rate of 0.33 while the Lunar Processing Lab's rate was slightly greater at 0.42. Two sampling events produced recovery rates that were more than one standard deviation above the mean recovery rate in the Meteorite Lab, with the first sampling in October 2017 nearly doubling the mean rate and the second sample was collected in October 2018. It is possible that this is a seasonal effect. The majority of isolates are from non-critical surfaces such as the floor or wall. Fungal isolates are primarily in the *Penicillium* genus while bacterial isolates are most often human-associated microbes such as *Staphylococcus sp.*

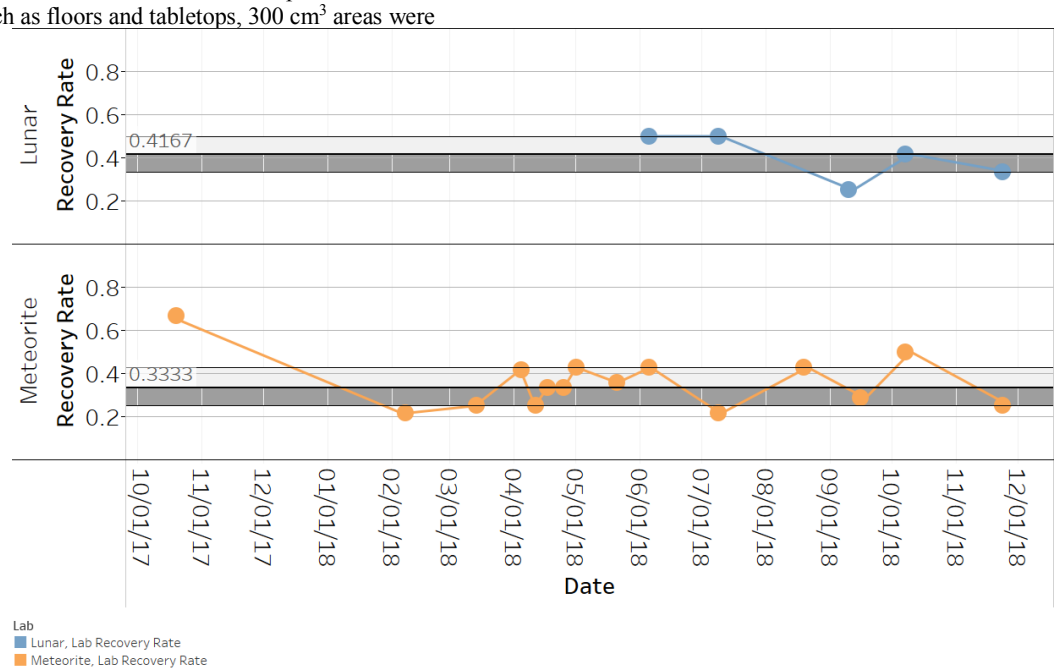

Figure 1: Recovery rates from the lunar curation lab (top) and meteorite curation lab (bottom).

Discussion: The low recovery rates on surfaces in the labs are most likely the result of a rigorous cleaning schedule. While aggressive anti-microbial or sporicidal cleaning solutions are not used in the labs, regular mechanical scrubbing with mild cleaning detergents seems to be effective at reducing microbial contamination. We are currently researching sporicidal cleaning solutions which do not

contaminate the collections with chemicals that interfere with scientific studies of the specimens.

The majority of bacterial isolates are most frequently associated with the human skin microbiome indicating they entered the lab through either direct skin contact with surfaces or as human-produced dust particles. Future work will include both culture-dependent and independent methods. Molecular analyses of bacterial and fungal communities in these samples will provide identification of culture-resistant microbes while characterization of fungal and bacterial isolates can determine the mechanisms of survival in these extreme environments as well as potential alteration of geological collections.

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