INSIGHTS FROM ROUTINE MICROBIOLOGICAL MONITORING OF AIR IN ASTROMATERIALS CURATION CLEANROOMS A. B. Regberg[1], C.P. Cohen[2], R.E. Davis[3], C.G. Kmiecik[2] and F. Mazhari[2], [1]Astromaterials Research and Exploration Science Division, NASA Johnson Space Center (aaron.b.regberg@nasa.gov), [2]Jacobs, JETS Contract, NASA Johnson Space Center, [3]Jacobs, JETS Contract, NASA Johnson Space Center

Introduction: NASA maintains nine separate cleanrooms at the Johnson Space Center to curate and preserve astromaterials samples. Routine microbial monitoring of the surfaces in these cleanrooms began in 2018[1]. Until recently, materials compatibility requirements prevented monitoring airborne biological particles in all but one of these cleanrooms. New astromaterials collections from carbon rich asteroids are more susceptible to biological degradation than previous collections. Therefore, it is important to monitor the bioburden in the air and on surfaces in these labs. Establishing a comprehensive microbial monitoring program will help inform the monitoring and curation plans for Mars sample return which will include samples that are extremely biologically sensitive[2,3]. In April of 2022 we began routinely collecting air samples in seven of the nine curation cleanrooms (Meteorite ISO 7 equivalent, Lunar ISO 6 equivalent, Stardust, Hayabusa2, OSIRIS-REx ISO 5 equivalent, and Genesis ISO 4 equivalent) using a sampling device that collects airborne biological particles on an electret filter instead conventional sampler that collect cells in a liquid media or onto organic rich Petri dishes. Electret is a generic term for electrostatically charged media. The charge on these materials increases particle trapping when compared to non-charged filters of similar thickness[4,5]. N95 respirators also use electret filters. This dry sample collection method allows us to meet materials requirements for all the curation labs and reduces the risk of inadvertently introducing contamination as part of our monitoring effort. This method also allows us to preserve a portion of each sample for DNA extraction and next generation sequencing. DNA sequencing helps us to characterize the portion of the cleanroom microbiome that we cannot culture. We will present the results of our first 8 months of monitoring, compare these results to particle counts in the labs and to measurements made directly onto Petri dishes when possible. We will also make recommendations for modifications to the sampling method to improve sampling efficiency and preserve diversity.

Methods: We used the Innovaprep Bobcat sampler to collect air samples in the astromaterials curation clean rooms. This sampler operates by pulling air through an electret filter at the rate of 200 L/min. The manufacturer recommends running the sampler for five minutes pausing for fifteen minutes and then sampling again for five minutes to ensure a representative sample. We collected two samples in every lab using this five minute on fifteen minute off method. One sample was collected for an hour (15 min. of active sampling or 3,000L of air) the other sample was collected overnight for approximately 17 hours (255 min. of active sampling or 51,000L of air).

After sample collection a pressurized mixture of 0.15% tween 20 in PBS (phosphate buffered saline) was used to rinse the particles from the filter into a volume of 6-7 ml for subsequent analysis. Petri dishes were inoculated with the eluent to detect and identify culturable bacteria according to previously published methods [1,6,7]. The remaining eluent was preserved for amplicon sequencing to detect and characterize bacteria, archaea and fungi present in the samples. We also performed 16S rRNA amplicon sequencing on three air samples collected in the ISO 7 equivalent advanced curation lab for 5, 15, and 360 minutes (six hours) respectively in order to test the effect of long duration sampling on DNA preservation. DNA was extracted using a Qiagen MagAttract PowerMicrobiome kit[8] and sequenced on an Illumina Miseq with a V3 reagent kit. The data was processed using the DADA2 and QIIME2 pipelines [9,10].

Results: Over the course of the initial 8 month sampling period, we collected 100 air samples and detected between 0 and 61 CFU (colony forming units) per air sample. The sample collected on 10/18/2022 from the meteorite processing lab had the largest number of CFU and contained the following organisms: *Penicillium sp.* (46 CFU), *Cladosporium sp*. (14 CFU) and, *Bacillus sp*. (1 CFU). We did not cultivate bacteria or fungi from 74 of the 100 samples collected. The mean number of CFUs per sample across all the labs is 2.6 The mean number of CFU for the one hour sampling time is 1.66 ($5.3*10^{-4}$ CFU/L). Overnight sampling increases the mean number of CFUs to 2.46 (($4.8*10^{-5}$ CFU/L). Fourteen of the fifty overnight samples contained culturable bacteria or fungi, while only eleven of the fifty hour long samples contained culturable microbes. The mean number of CFU detected correlates with the ISO class of the lab with the ISO 7 equivalent lab having ten times more CFU (6.7 CFU per sample) on average than the ISO 5 equivalent labs (Stardust 0.05 CFU per sample, Hayabusa2 0.94 CFU per sample, OSIRS-REx 0.61 CFU per sample). Samples containing culturable

microbes did not correlate to high particle counts in the labs.

Preliminary results from amplicon sequencing of the 16S rRNA gene appear to show decreases in the amount of detectable DNA and diversity at longer sampling times. The five minute and fifteen minute samples contained 6,134 reads from 0.418 ng/µl of DNA and 6,969 reads from 0.316 ng/µl of DNA respectively. The six hour sample contained 3,582 reads from 0.277 ng/µl of DNA . We identified 25 ASV (amplicon sequencing variants) in the five minute sample, 20 ASV in the fifteen minute sample and 10 ASV in the six hour sample. ASV are thought to correspond to different species or strains of bacteria[9]

Discussion and Future Work The Bobcat air sampler appears to be an effective way to collect biological samples from astromaterials curation clean rooms without introducing unwanted organic contaminants associated with impactor style samplers. Dedicated biological air sampling is necessary because high particle counts do not always appear to correlate with large numbers of culturable organisms. This result implies that most of the particles in these labs are not viable cells.

Most of the samples collected during the 8 months contained no culturable bacteria or fungi indicating that the air in the labs is relatively free of bioaerosols. When culturable organisms are present, sampling for a longer period results in a slightly larger average number of CFU but a lower number of CFU/L. Preliminary data from amplicon sequencing suggests that sampling for longer time periods may decrease the amount of DNA present in samples and artificially lower the diversity while biasing the results towards spore forming bacteria from the *Bacillus* genus (Fig 1). Further work is needed to optimize the sampling time for these cleanrooms.

References

1. Regberg, A. B. *et al. 49th Lunar and Planetary Science Conference* 2056 (2018).
2. McCubbin, F. M. *et al. Space Sci Rev* **215**, (2019).
3. Meyer, M. A. *et al. Astrobiology* (2021)
4. Thakur, R., Das, D. & Das, A.. *Separation & Purification Reviews* **42**, 87–129 (2013).
5. Barrett, L. W. & Rousseau, A. D. *American Industrial Hygiene Association Journal* **59**, 532–539 (1998).
6. Davis, R. E., *et al. 50th Lunar and Planetary Science Conference 2019* 2880 (2019).
7. Regberg, A. B. *et al. 53rd Lunar and Planetary Science Conference* . **2678**, 1686 (2022).
8. Qiagen. (2018).
9. Callahan, B. J. *et al. Nature Methods* **13**, 581–583 (2016).
10. Bolyen, E. *et al. Nature Biotechnology* 1 (2019)

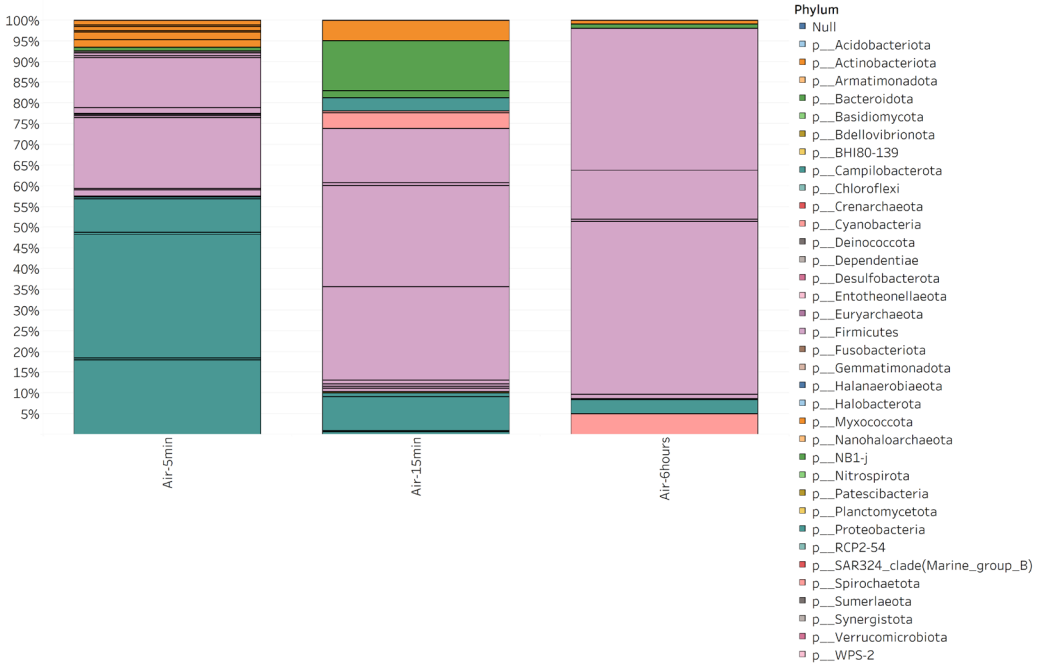

Figure 1: Relative abundance of 16S rRNA sequences from bacterial phyla for three different air samples. Bacterial diversity decreases with longer sampling times. Sequences from the Firmicutes phylum (pink bars) are more abundant in samples with longer collection times.