MICROBIAL MONITORING OF NEW CLEANROOMS USED TO CURATE ASTROBIOLOGICALLY RELEVANT ASTEROID SAMPLES FROM BENNU AND RYUGU A. B. Regberg[1], K.K. Allums[2], C.L. Castro[3], R.E. Davis[4], R.C. Funk[2], N.G. Lunning[1], F. Mazhari, K[2]. Nakamura-Messenger[1], K. Righter[1], C.J. Snead[4], and F. M. McCubbin[1] [1]Astromaterials Research and Exploration Science Division, NASA Johnson Space Center, [2]Jacobs, JETS Contract, NASA Johnson Space Center,.[3]KBRwyle, NASA Johnson Space Center, [4]Texas State University, Johnson Space Center

Introduction: NASA has constructed two new cleanrooms to house materials from the OSRIS-REx and Hayabusa2 missions to the asteroids Ryugu (162173) and Bennu (101955), respectively. In accordance with standard astromaterials curation practices, these cleanrooms will be monitored for particulate contamination and maintained to ISO 5 equivalent standards[1]. Since the samples in these collections are expected to contain prebiotic organic compounds that may help explain the origin of life on Earth, these labs will also be monitored for organic and biological contamination[2]. Samples from Ryugu arrived on Earth in December, 2020. After basic characterization in Japan, NASA received a subset of these samples at the astromaterials curation facility in Houston in December of 2021. OSIRIS-REx is expected to return samples in September, 2023. Here we present preliminary microbial monitoring results from monthly monitoring of these new labs and the connected microtomy and staging areas that support them, as they are being commissioned. We also compare these results to baseline values for other astromaterials curation labs. We will also briefly describe additional cleaning efforts employed to reduce the bioburden in these new cleanrooms.

Methods: Microbial samples were collected from surfaces using a dry macrofoam swab (Puritan Brand 2518051PFRNDFD). Swabs were also opened in the lab but not touched to any surfaces to function as negative controls. Samples and controls were processed inside a class II biosafety cabinet to avoid inadvertent cross contamination. The swabs were suspended in 15 ml of PBS (Phosphate Buffered Saline) and vortexed for 20 seconds to remove cells from the swab surface. The PBS was used to inoculate Petri dishes filled with TSA (Tryptic Soy Agar), Blood Agar, or Reasoners 2 agar to check for microbial growth. Each plate was inoculated with 0.1 ml of PBS. The TSA and blood agar plates were incubated at 35˚C and the Reasoners 2 agar plates were incubated at 25˚C for seven days. Petri dishes filled with Potato dextrose agar, Saboraud dextrose agar, or Saboraud dextrose agar with 0.1 mg/ml of chloramphenicol, an antibiotic, were used to check for fungal growth. These plates were inoculated with 0.3 ml of PBS and incubated at 30˚C. The remaining PBS was frozen at -80 ˚C for DNA sequencing. After incubation, isolates were counted and reisolated for identification. Isolates were identified using the VITEK2[3] system or by sequencing a portion of the 16S rRNA gene for bacteria or the ribosomal internal transcribed spacer (ITS) for fungi. Sequencing was performed with an ABI 3500 Sanger sequencer.

Results: During our initial sampling, six of the seven sites sampled (86%) displayed bacterial or fungal growth. Samples collected from the staging areas and microtomy labs are not included in this calculation since those areas are maintained at a lower ISO 7 equivalent cleanliness standard. A month later, only three of the seven sites (43%) displayed bacterial growth. No fungal growth was detected in the second sampling. Since new equipment had been introduced to the Hayabusa2 lab since the first round of sampling, an additional three sampling sites were included in the second round of sampling. None of these sites displayed microbial growth. These sites will be included in all future sampling efforts.

Bacterial isolates have been identified from the following genera at multiple time points: *Micrococcus, Staphylococcus,* and *Bacillus.* Isolates from the genera: *Microbacterium, Nocardioides, Methylocystis,* and *Microvirga* were identified in the initial sampling, but were not present at later time points. Identification of fungal isolates is in progress. Results are summarized in Table 1.

Discussion: The recovery rate or percentage of positive samples[4] was initially 86%, which is higher than the median recovery rate for comparable ISO 5 equivalent curation labs like Stardust (33%), Hayabusa (33%), and Cosmic Dust (50%). However, after a month of operation, the recovery rate for these same sites decreased to 43%, which is similar to what we observe in comparable curation cleanrooms with no microbial control requirements. Adding in the new sampling sites further decreases the recovery rate to 30%. With the reduction in recovery rate, we also observed a decrease in microbial diversity. At the first time point, we observed at least 10 different bacterial species and at least two different fungi. This is a higher diversity than the median values for comparable ISO 5 equivalent labs (2-4 isolates per sampling event). After the second sampling, we observed at least 4 bacterial species and no fungi, which is more consistent with comparable labs. We expect the recovery rate and diversity in both labs to continue to decrease as routine operation

continues. We will use ultrapure hydrogen peroxide to disinfect equipment and work areas prior to opening any sample containers.

Most of the bacterial and fungal isolates were detected on samples from the cleanroom floors. This is consistent with baseline results from other curation labs. Organisms from the genera *Bacillus, Staphylococcus,* and *Micrococcus* that were repeatedly detected are common in cleanrooms and on human skin[5,6]. These organisms are generally thought to be introduced when people enter the cleanroom. *Microbacterium, Nocardioides,* and *Microvirga* have also previously been identified in astromaterials cleanrooms, but not as frequently as *Bacillus, Staphylococcus,* and *Micrococcus*. *Methylocystis* is a novel genus in the astromaterials cleanrooms, but it was identified with low accuracy (93% match in the sequenced region of the 16S rRNA gene) and further work is needed to confirm this identification. *Microbacterium* is a diverse genus with isolates identified from terrestrial and aquatic sediments. Some species of *Microbacterium* are capable of degrading complex organic compounds found in crude oil. The presence of these bacteria in the OSIRIS REx and Hayabusa2 cleanrooms should be closely monitored. *Methylocystis* is a genus of methanotrophic bacteria capable of oxidizing methane. If this identification proves to be correct and it is detected again, it should be closely monitored as well.

Under nominal operating conditions, samples should not ever encounter the cleanroom floor or other high traffic areas. If we observe an increase in the bioburden in sensitive work areas that appears to be influenced by organism transfer from high traffic areas like the floors, we can employ additional hydrogen peroxide treatments to disinfect high traffic areas. Routine microbial monitoring of these labs will ensure that NASA's astromaterials collections remain pristine and useful for scientific study.

Table 1. Sampling Locations and Colony Counts

Lab - Location	Bacterial CFU[a] 11/2/2021	Fungal CFU 11/2/2021	Bacterial CFU 12/13/2021	Fungal CFU 12/13/2021
H2[b]-Floor	4	8	1	0
H2-staging pass through	3	0	0	0
H2-microtomy pass through	TNTC[c]	0	0	0
H2 Microscope 1	NA	NA	0	0
H2 Microscope 2	NA	NA	0	0
H2-Table	NA	NA	0	0
OREX[d]- microtomy pass through	0	0	6	0
OREX – Anteroom pass through	0	0	0	0
OREX – Floor	1	2	0	0
OREX Witness Foil Table	3	0	1	0
Staging-Floor	16	0	15	0
Microtomy-Floor	3	0	2	0

a: CFU = Colony Forming Unit
b: H2 = Hayabusa2 Lab
c: TNTC = too numerous to count
d: OREX = OSIRIS-REx Lab

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