A METHOD TO REDUCE BIOBURDEN IN ASTROMATERIALS CURATION FACILITIES WITHOUT INTRODUCING UNWANTED CONTAMINATION A. B. Regberg[1] , C.L. Amick[2], R. E. Davis[2], E.K. Lewis[3], F. Mazhari[2], J.L. Mitchell[1], D.L. Owens[2] and, F. M. McCubbin[1] [1]Astromaterials Research and Exploration Science Division, NASA Johnson Space Center, 2101 NASA Parkway, Houston TX, 77058 [2] Jacobs, JETS Contract, NASA Johnson Space Center, 2101 NASA Parkway, Houston TX 77058, [3]Texas State University, Johnson Space Center 2101 E NASA Pkwy Houston, TX, 77058, USA

Introduction: NASA curates its Astromaterials collections in cleanrooms that are carefully monitored for particulate, inorganic and trace metal contamination. Current sample collections are not particularly susceptible to organic contamination or biological alteration. However, new collections like those from the OSIRIS-REx and Hayabusa2 missions will have organic contamination requirements and are susceptible to biodegradation. It will be necessary to sterilize or at least disinfect curation labs, as well as tools and equipment in a manner that does not introduce additional contamination and does not affect the samples[1]. Current curation cleaning procedures utilize isopropyl alcohol, which offers some bioburden reduction, but is not effective against spore-forming bacteria or fungal spores[2]. We present a modified disinfection method that uses ultrapure hydrogen peroxide to reduce bioburden inside curation labs and glove boxes without introducing contamination or damaging curation equipment. We tested this method in the meteorite processing lab as well as on a glovebox being cleaned for use in processing ANGSA (Apollo Next Generation Sample Analysis) samples and present the results of those tests. We discuss the limitations of this method and describe potential situations in which it will not be applicable.

The CDC (Center for Disease Control) guidelines for disinfection and sterilization in healthcare facilities discusses over 15 different methods for reducing bioburden in hospital settings[3]. The most common method, steam sterilization, is well suited to sterilizing curation processing tools but cannot easily be used to sterilize cleanroom surfaces or large equipment like gloveboxes. Chemical sterilization with bleach (NaOCl) is also a common strategy in healthcare and pharmaceutical settings that presents material compatibility issues as well as serious inorganic contamination concerns for curation facilities. Introducing a new source of Na and Cl into curation labs is not acceptable. Other chemical methods like ethylene oxide, formaldehyde, iodophors and quaternary ammonium compounds could introduce organic and inorganic contamination. We chose to focus on hydrogen peroxide because it is generally compatible with commonly used curation materials like stainless steel, aluminum and Teflon and because it decomposes to oxygen and water. The CDC guidelines for hydrogen peroxide specify using a 7.5 wt% solution at 25˚C with a contact time of 30 minutes for high level disinfection and 6 hours for sterilization. High level disinfection is defined as a technique that will kill all microorganisms except large numbers of bacterial spores [3].

Methods: We prepared a solution of 7.5 wt% hydrogen peroxide from a stock solution of ultrapure 30 wt% peroxide (JT Baker) and curation grade ultrapure water. This ultrapure water is already used in curation cleaning procedures and thus is not considered and additional source of contamination. We conducted a materials compatibility test by exposing un-anodized and anodized 6061 T6 Al alloy to the peroxide solution for up to six hours, and periodically inspecting the surfaces for visible defects. We used this peroxide to disinfect the floor of the meteorite processing lab and the interior of a curation glovebox by exposing these surfaces to the peroxide solution for 30 min. Portions of the surfaces were swabbed (300 cm^2) with a dry macrofoam swab before (Puritan Brand 2518051PFRNDFD) and after peroxide treatment to collect microbes present on the surfaces. Microbes were extracted by sonication from the swab into 15 ml of PBS (phosphate buffered saline) and inoculated onto the following media: TSA (tryptic soy agar) BA (blood agar), R2A (Reasoners 2 agar), Potato Dextrose Agar, Saboraud Dextrose Agar and Saboraud Dextrose Agar with 0.1 mg/ ml chloramphenicol. Four TSA plates and two BA plates were inoculated with 0.1 ml of PBS each and incubated at 35℃ and 37℃ for 48 hours. Two R2A plates (0.1 ml of PBS each) were incubated at 25℃. The remaining plates were inoculated with 0.2ml of PBS and incubated at 30˚C for seven days. After incubation bacterial and fungal isolates were counted and transferred to new plates for identification using the VITEK2[4] automated system or by sequencing a portion of the barcode gene (16S rRNA for bacteria, small subunit gene for fungi) on an ABI 3500 Sanger sequencer. Negative controls consisted of swabs that were opened in the sampling environment and analyzed alongside the experimental samples.

Results: A 6-hour exposure to hydrogen peroxide resulted in visible pitting on un-anodized 6061 Al, but not on anodized surfaces. No visible pitting occurred

after a 30-minute exposure on either surface. Therefore, we decided to limit our experimental tests to 30 min. exposures. 17 bacterial CFU (colony forming units) representing 4 distinct organisms were isolated from the meteorite processing lab floor prior to hydrogen peroxide treatment. We were unable to culture any organisms after peroxide treatment. In the glovebox, we were able to culture three bacterial CFU representing three distinct species, including a spore forming bacterium prior to disinfection with peroxide. After the peroxide treatment we were unable to culture any organisms. Routine monitoring of the meteorite processing lab and the glovebox did not indicate any increase in unwanted inorganic contamination after these peroxide treatments.

Discussion: A 30-minute treatment with 7.5 wt% peroxide appears to be an effective method for reducing bioburden on typical cleanroom surfaces. The method does not introduce unwanted organic or inorganic contamination and is compatible with commonly used curation materials like stainless steel, Teflon and anodized aluminum alloys. Special care should be taken with un-anodized aluminum. Because prolonged exposure to hydrogen peroxide can cause pitting on this material. We recommend using this method to disinfect curation labs and equipment when biological alteration is a concern. This method is effective at room temperature and cannot be used to disinfect labs and equipment where the ambient temperature is $\leq 0^{\circ}C$. Astromaterials samples should be removed from the area where disinfection is to occur. Hydrogen peroxide is a powerful oxidizing agent and will react with any organic carbon present in the sample.

References: **[1.]** McCubbin, F. M. *et al. Sp. Sci Rev* (2019) doi:10.1007/s11214-019-0615-9. **[2.]** Mogul, R. *et al. Astrobiology* 18, ast.2017.1814 (2018). **[3.]** Rutala, W. A. & Weber, D. J. *Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008*. **[4.]** Pincus, D. H. in *Encyclopedia of Rapid Microbiological Methods* (2005).