THE MICROBIAL CONTAMINATION STATE OF AS-FOUND ANTARCTIC METEORITES. [1]Fries M., [2]Harvey R., [3]Jull A.J.T., [4]Steele A., [5]Wainright N., and the ANSMET 07-08 Team. [1]Planetary Science Institute, Tucson AZ, [2]Case Western Reserve University, Cleveland, OH, [3]University of Arizona, Tucson, AZ, [4]Carnegie Institute for Science, Washington, DC, [5]Charles River Laboratories, Wilmington, MA. *fries@psi.edu*

Summary: Microbial contamination was measured in Antarctic meteorites to test the hypothesis that these meteorites are sterile or nearly so in their as-found condition. Meteorites were collected aseptically and then sampled and analyzed in a sterilized glove box. Extracts from these measurements were kept frozen until the measurements could be repeated in a laboratory. Laboratory measurements proved to be consistent with field measurements, establishing the validity of the measurements obtained in the field. Two measurement techniques were used: limulus amebocyte lysate (LAL) assay featuring single-cell sensitivity to gram-negative microbes, and adenosine triphosphate (ATP) luminometry which is sensitive to the metabolism of all cells. The body of measurements indicates that *Antarctic meteorites are sterile in their as-found condition*, with the caveat that this suite of measurements is less sensitive to the presence of gram-positive microbes such as fungi. The same measurements performed on meteorites collected with the standard ANSMET protocol indicate that microbial contamination is introduced during collection. Realistically, however, microbial contamination should be viewed as an inevitability to be minimized. Eliminating it completely would strain available resources for little real gain. Furthermore, aseptic collection of Antarctic meteorites is a very time-consuming process that would have a severe negative effect on the number of Antarctic meteorites available for research. Replacement of the existing protocol is not warranted, but rather any scientific study where microbial contamination is a factor should include quantitative analysis of the contamination state of that specific sample.

Figure 1: Aseptic meteorite collection. The collector is wearing a full-body clean-room garment, boot covers, sterile gloves, and a face-mask. The meteorite is handled with sterilized tongs and stored in a plasma-cleaned Al container with witness plates. The container is sealed in a furnace-sterilized Al bag wrapped in Al foil. Collection is performed downwind from the meteorite.

Methods: Six meteorites were collected aseptically (Figure 1) during collection sessions specified for this activity. All snowmobiles approached from downwind and were turned off when a candidate meteorite was sighted. Of the six meteorites, three were ordinary chondrites suitable for use in this study. Sufficient reagents were available to supplement the study by analyzing three meteorites collected via the typical ANSMET protocol. Three ordinary chondrites were selected at random from the box of meteorites collected that season, with the permission and oversight of the Meteorite Working Group chairman. These meteorites were sampled and analyzed in a single session. The glove box was sterilized using a proven field sterilization protocol [1] and measurements were performed in a warm tent with dry Antarctic air pumped into the glove box through a filter/desiccator apparatus (Figure 2). 5g of material was separated from each

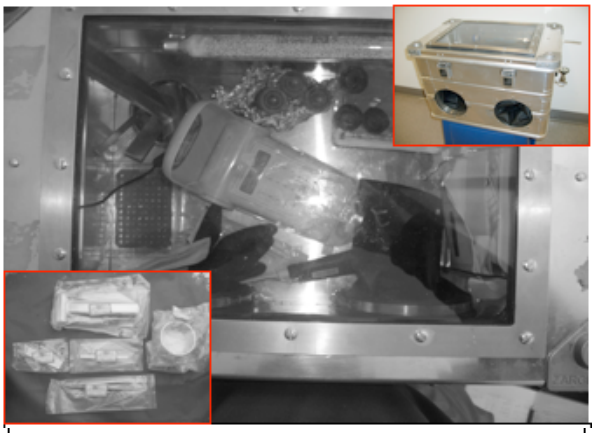

Figure 2: Sterilized metal glove box used for measurements. Large image: Top-down view showing samples, air handling filter/desiccator (top) and LAL instrument (center). Inset upper right: Overview of glove box. Inset lower left: Sterilized, sealed meteorite sampling tools provided by the Astromaterials Curation Laboratory, NASA Johnson Space Center.

meteorite and crushed, 5 mL of pyrogen-free water was added to each, the mixture was vortexed for 10 minutes and allowed to settle, and supernatant fluid was pipetted off for LAL and ATP measurements. No two sample containers were opened at the same time, and the glove box was cleaned between samples. A round of measurements were also performed on witness plates. After the measurements were completed, the collection of meteorite/water aliquots were frozen and remained so until re-analyzed in the microbiology laboratory at the Geophysical Laboratory of the Carnegie Institute of Science. All LAL and ATP measure-

ments were repeated to test the validity of the field measurements.

Results and Discussion: ATP measurements for both field and laboratory measurements (Figures 3 and 4, respectively) show no detectable microbial metabolism is occurring in any sample. This does not indicate that the samples are sterile, but rather that any microbes present are quiescent.

LAL results consistently produced zero values for microbial abundance in the meteorites collected by aseptic procedures (Figures 3 and 4). Field measurements of the meteorites collected using the ANSMET protocol, however, produced non-zero values for all three meteorites tested (Figure 3). Repeat measurements in the laboratory show that two of those three show zero values and the meteorite that produced a maximum-range response in the field (MIL 07114) retains a high value (Figure 4). The diminished response is most probably the result of some sample degradation in transport, which highlights the need for field measurements for this type of study.

Results of this study show that meteorites found on the Antarctic ice are sterile to within the limits of this study.

The evidence of microbial contamination in meteorites collected via the ANSMET protocol does not immediately require that the protocol be amended. The aseptic collection protocol required bulky equipment and supplies and *took roughly ten times as long as collection using the ANSMET protocol*. Applying aseptic collection procedures would effectively cut the number of meteorites collected roughly ten-fold, and would not prevent meteorites from accruing contamination once they leave Antarctica. In balance, the current protocols are in good balance with the need for relatively large numbers of new and/or unusual meteorites for meteorite research.

References:

[1] Eigenbrode J. *et al*, *Astrobiology* **9** (2009) 455-465.

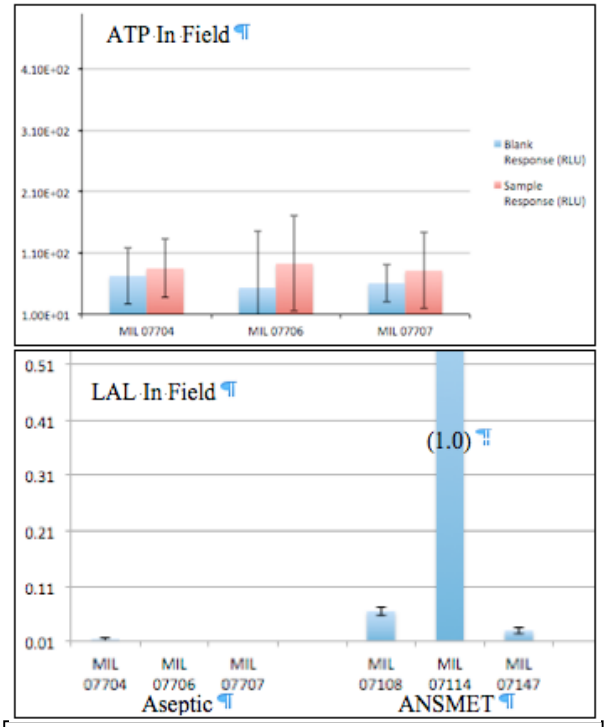

Figure 3: LAL and ATP measurements in the field. ATP measurements (TOP) show that aseptically collected meteorites feature instrument values statistically indistinguishable from background values. ATP was unavailable for meteorites collected with the ANSMET protocol. LAL measurements (BOTTOM) show zero values for aseptically collected meteorites but non-zero values for all three meteorites collected via the ANSMET protocol.

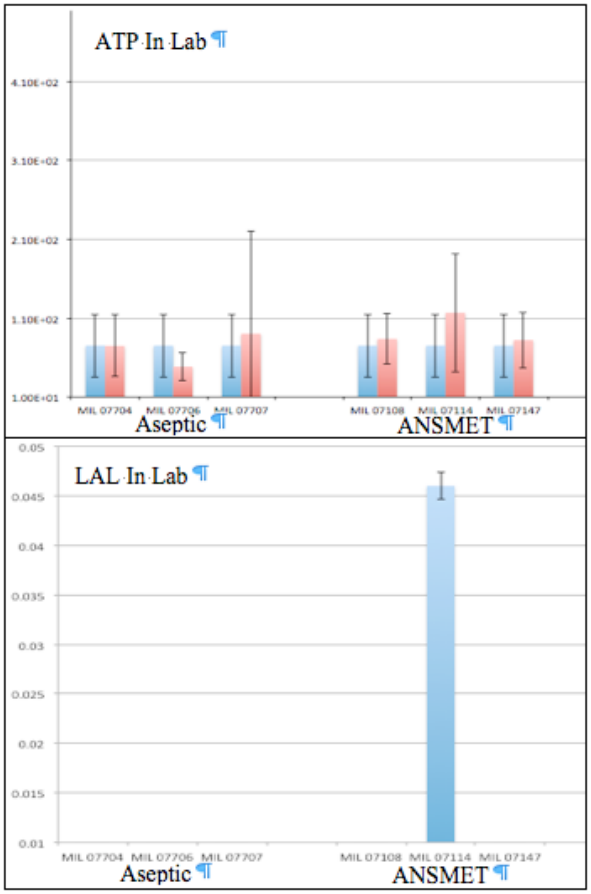

Figure 4: ATP and LAL measurements repeated in the laboratory. ATP measurements (TOP) are consistently statistically indistinguishable from background values. LAL results (BOTTOM) imply some degradation of the samples such that only one of the three ANSMET protocol samples retains a positive value. This is a reasonable result. The combination shows that any contaminating microbes present are not actively metabolizing.