**LESSONS FROM ASTROBIOLOGICAL PLANETARY ANALOGUE EXPLORATION IN ICELAND: BIOMARKER ASSAY PERFORMANCE AND DOWNSELECTION.** Diana Gentry[1], Elena Amador[2], Morgan Cable[3], Thomas Cantrell[4], Nosheen Chaudry[5], Thomas Cullen[5], Zachary Duca[4], Jessica Kirby[5], Malene Jacobsen, Heather McCaig[3], Gayathri Murukesan[6], Vincent Rennie[7], Edward Schwieterman[2], Adam Stevens[7], George Tan[4], Chang Yin[8], Amanda Stockton[4], David Cullen[5], and Wolf Geppert[8]. [1]Biospheric Science Branch, NASA Ames Research Center, MS 245-4, Moffett Field, CA 94035, USA diana.gentry@nasa.gov [2]Astrobiology Program, University of Washington, 4000 15th Ave NE, Seattle, WA 98195, USA [3]NASA Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Dr, Pasadena, CA 91109, USA [4]Georgia Institute of Technology, School of Chemistry & Biochemistry, 901 Atlantic Drive, Atlanta, GA 30332, USA [5]School of Engineering, Cranfield University, College Road, Cranfield, MK43 0AL, Bedfordshire, UK [6]University of Turku, Department of Biochemistry/Biochemistry, 20014 Turun Yliopisto, Finland [7]University of Edinburgh, James Clerk Maxwell Building, Peter Guthrie Tait Road, Edinburgh EH9 3FD, UK [8]Stockholm University, Astrobiology Centre, SE - 106 91, Stockholm, Sweden.

**Introduction:** We have conducted four expeditions to Icelandic Mars analogue sites in which an increasingly refined battery of physicochemical measurements and biomarker assays were performed, staggered with scouting of further sites. A further three expeditions are planned to complete the study; intermediate results are reported here.

Planetary exploration is currently reliant on robotic missions. Such missions are typically highly constrained both in the number of different assays they can perform and in the number of repetitions of each assay that can be performed. This is particularly true for the 'wet' assays often required for life or biomarker detection, such as those used on the Mars Science Laboratory's Science at Mars instrument suite.

Understanding the sensitivity of biomarker assays to the local physicochemical environment, and the underlying spatial distribution of the target biomarkers in 'homogeneous' environments, can increase mission science return in the face of this limit in two ways. Firstly, *a priori* information about biomarker distribution can be used to set the number and location of sample sites required. Secondly, understanding the impact of those environmental parameters that can be determined through remote sensing can be used to choose geographical regions where a small number of sample replicates are likely to be representative of the given area. Lastly, science return can be increased if more broadly representative *in situ* assays can performed initially and the results used to rapidly downselect local sites for further, more sensitive analysis.

Given the extremely limited opportunities for planetary *in situ* science, information from terrestrial analogues is critical for achieving these goals.

**Methods:** Completed expeditions took place in 2012 (location scouting and field assay use testing), 2013 (sampling of two major sites with three assays and observational physicochemical measurements), 2015 (repeat sampling of prior sites and one new site, scouting of new sites, three assays and three instruments), and 2016 (preliminary sampling of new sites with analysis of returned samples).

*Sites.* Target sites were geologically recent basaltic lava flows, which have a long history as astrobiological Mars analogue sites [1]. Sample sites were arranged in hierarchically nested grids at 10 cm, 1 m, 10 m, 100 m, and >1 km order scales, subject to field constraints.

The Fimmvörðuháls (63º 38' N, 19º 26' W) lava field, sampled in 2012, 2013, and 2015, formed from a basaltic effusive eruption associated with the 2010 Eyjafjallajökull eruption; the field site is located in a saddle between the larger Eyjafjallajökull and Myrdalsjökull volcanic structures. The Eldfell (63º 25' N, 20º 14' W) site, sampled in 2012 and 2013, associated with the Vestmannaeyjar volcanic system, formed from both effusive and explosive alkali basalt eruptions on the island of Heimaey in 1973. Mælifellssandur (63º 49' N, 19º 09' W), sampled in 2015, is an older gravel plain to the north of the Myrdalsjökull glacier with extensive water activity. Holuhraun (64° 51' N, 16° 50' W), sampled in 2016, was formed from 2014 fissure eruptions just north of the large Vatnajökull glacier.

*Assays and instruments.* Assays were intended to represent a diversity of potential biomarker types (biomass, bioavailable energy, intact cells, etc.) rather a specific mission science target, and were selected to reduce laboratory overhead, limited consumables compatibility, and rapid turnaround. All analytical work was performed *in situ* or in a field laboratory within a day's travel of the field sites unless otherwise noted.

Adenosine triphosphate (ATP) quantification was performed with the Roche ATP Bioluminescence Assay Kit HS II following the manufacturer's recommendations modified as in [2]. Direct cell quantification using fluorescence microscopy was performed on sample

wash stained with SYBR Gold Nucleic Acid Gel Stain (Invitrogen) as described in [3], with minor modifications. Relative quantification of fungal, bacterial, and archaeal DNA was performed after sample DNA extraction using the qPCR technique described in [4]. Specific primers, instruments, and other information have been published in [5]. Absolute DNA quantification and community sequencing were performed on returned samples starting in 2015 as well.

Mineralogical, chemical, and physical measurements and observations were intended to complement biomarker assay results. All sites chosen were 'homogeneous' at the high level of apparent color, morphology, moisture, and grain size. Temperature readings were taken in 2012 and 2013. pH readings of sample wash were taken from 2015 onwards. Raman spectroscopy and reflectance spectroscopy were performed on selected samples in 2015 and 2016.

*Analyses.* We determined several measures of spatial distribution and variability of biomarkers: unbiased sample variance, *F*- and pairwise *t*-tests with Bonferroni correction, and the non-parametric *H*- and *u*-tests. The non-parametric tests were included to examine the effect of *a priori* assumptions about biomarker distribution. All assays results were then compared using the non-parametric Spearman's rank test to characterize their degree of correlation.

**Results and Observations:** We have demonstrated the feasibility of performing ATP quantification and qPCR analysis in a field-based laboratory with single-day turnaround. The ATP assay was generally robust and reliable and required minimal field equipment and training to produce a large amount of useful data. DNA was successfully extracted from all samples, but the serial-batch nature of qPCR significantly limited the number of primers (hence classifications) and replicates that could be run in a single day. Fluorescence microscopy did not prove feasible under the same constraints, primarily due to the large number of person-hours required to view, analyze, and record results from the images; however, this could be mitigated with higher-quality imaging instruments and appropriate image analysis software.

Temperature and pH measurements were straightforward. Reflectance spectroscopy initially did not yield significant information due to the darkness of the basaltic tephra comprising most of our samples, but was improved after re-calibration. Raman spectroscopy in our field laboratory had challenges as well, including limited control of background light, and was changed to use on returned samples in 2016.

Fimmvörðuháls has proved to be a reliable and high-yield field site, accessible over multiple field seasons, providing several areas with similar, undisturbed geology that are both a short enough hike from the nearest vehicle access to allow transport of field equipment yet still with relatively limited recreational activity. Eldfell had significantly higher anthropogenic disturbance, and, requiring ferry travel, was subject to weather limitations. Mælifellssandur was initially promising due to its easy access, but as a commonly flooded area, the amount of transported material present made finding enough sample sites that met our definition of 'homogeneous' difficult. The Holuhraun field, which did not exist prior to our 2015 expedition, appears similar to Fimmvörðuháls and will be a focus going forward.

The ATP assay appears to be significantly more sensitive to small changes in sampling location and environment than qPCR or fluorescence microscopy. Bacterial and archaeal DNA content were more consistent at the smaller scales, but similarly variable across more distant sites. Conversely, cell counts and fungal DNA content have significant local variation but appear relatively homogeneous over scales of > 1 km. ATP, bacterial DNA, and archaeal DNA content were relatively well correlated at many spatial scales, but we will examine additional physicochemical variables in our future expeditions to determine if dependence on previously non-observed parameters explains the lack of consistent correlation seen overall.

**Conclusions:** Over the course of our expeditions, we have refined the balance between in-field and 'sample return' strategies for each of our assays and instruments to improve science yield. We have dropped fluorescence microscopy as an assay, increased the field focus on the time-sensitive ATP quantification assay, and changed our in-field protocols for DNA quantification and qPCR to post-expedition sample analysis at our respective institutions. This has allowed the addition of community sequencing, performed on the same extracted DNA aliquots, to our analysis battery. Similarly, although we were able to obtain some Raman results in our field laboratory, we have changed from using field instruments to analyzing returned samples, which has allowed the addition of powder XRD as a mineralogical tool. We are also focusing now primarily on Fimmvörðuháls, for which we have three years of data, and two potential sites within the Holuhraun field.

**References:** [1] Cousins C. R. *et al* (2013) *J. Volcanol. Geotherm. Res., 256*, 61-77. [2] Barnett M. J. (2012) *Applied Microbiology*, 10.1016/B978-0-12-394382-8. [3] Kepner R. L and Pratt J. R. (1994) *Microbiol. Rev., 58(4),* 603-615. [4] Livak, K. J. and Schmittgen, T. D. (2001) *Methods*, *25(4)*, 402-408. [5] Amador, E. S. *et al.* (2015) *Planetary and Space Science*, *106* 1-10.