AN INTEGRATED SYSTEM FOR LABELING AND DETECTION OF BIOLOGICAL MOLECULES IN MARS ANALOG REGOLITH USING AN ANTIBODY MICROARRAY. J. Maule[1], J. Toporski[1], N. Wainwright[2] and A. Steele[1], [1]Geophysical Laboratory, Carnegie Institution of Washington (j.maule@gl.ciw.edu), [2]Marine Biological Laboratory, Woods Hole, MA.

Protein microarray analysis has had a major impact in modern biology and medicine by enabling detection of hundreds of proteins (and other molecules) using extremely small sample volumes [1-4]. Protein microarrays are printed on a glass slide using a robot and are only a few millimeters in diameter. Each consists of a grid of several hundred spots (100-200μm-diameter) of protein. Protein microarrays can be divided into two categories: target protein arrays [5] and antibody (Ab) microarrays [6]. Target protein microarrays have been used to study protein interactions with pharmaceutical drugs [7], enzyme substrates [8] and antibodies [9, 10]; the latter are examples of "antigen (Ag) microarrays". Ab microarrays are arrays printed with antibodies, each of which binds to and recognizes a specific molecule termed the "antigen". Some antibodies printed in a microarray format can detect antigens at concentrations below 1ng/ml [6]. Used most extensively for the analysis of human proteins in biomedical applications [11-15], this type of array has not been used for the detection of microbial or viral antigens in environmental samples [although microbial/viral *antigen* microarrays have been used for serodiagnosis [16]]. We have proposed that such a microbe-specific Ab microarray would provide a useful tool for *in situ* detection of biological molecules in space (due to its low mass, broad search capability and small sample required).

Previous *in situ* analysis of another planet by instruments specifically designed to search for life occurred during the NASA Viking Missions on Mars in the 1970s. Three separate biology experiments were carried on each Viking lander spacecraft, each a test for *living microorganisms* [17]. These included: 1) Exposure of regolith to ^{14}C-labeled CO_2 gas [to detect carbon fixation (Pyrolitic Release, PR)]; 2) Exposure of regolith to ^{14}C-labeled liquid nutrients [to detect metabolism (Labeled Release, LR)]; and 3) Exposure of regolith to unlabeled nutrients followed by monitoring the production or uptake CO_2, N_2, CH_4, H_2, and O_2 using a gas chromatograph (Gax Exchange, GEX). In addition, a gas chromatograph/mass spectrometer (GCMS) performed a "molecular analysis" experiment to search for organic compounds in the upper surface layers. While decomposition of ^{14}C-nutrient to ^{14}C-gas occurred during the LR experiment, no organics were detected by the GCMS, leading to an initial conclusion that these results were due to non-biological phenomena [18, 19]. Others suggested that an alternative biological explanation for the LR results could not be ruled out, given that the GCMS was relatively insensitive and would not have been able to detect below 10^6 bacteria/gram regolith [20, 21]. However, a more recent study has attributed the Viking observations to superoxide radical ions, formed on Mars analog mineral surfaces following exposure to ultra-violet radiation in a simulated Mars atmosphere [22].

In contrast to Viking's tests for *living microorganisms*, we propose Ab microarray analysis as a tool for the *in situ* detection of molecules derived from microorganisms, *which may be dead or alive at the time of testing*. An integrated method and results are presented for the labeling of antigens in a Mars analog regolith and subsequent detection by Ab microarray.

References: [1] Perrin, A., et al., (2003) Anal Biochem. **322**(2): p. 148-55. [2] Urbanowska, T., et al., *(2003)* Cell Biol Toxicol. **19**(3): p. 189-202. [3] Woodbury, R.L., S.M. Varnum, and R.C. Zangar, (2002) J Proteome Res. **1**(3): p. 233-7. [4] Yoshitake, Y., et al., *(2004)* Clin Cancer Res. **10**(19): p. 6437-48. [5] MacBeath, G. and S.L. Schreiber. Science, 2000. **289**(5485): p. 1760-3. [6] Haab, B.B., M.J. Dunham, and P.O. Brown, *(2001)* Genome Biol. **2**(2): p. RESEARCH0004. [7] Kumble, K.D., *(2003)* Anal Bioanal Chem. **377**(5): p. 812-9. [8] Rychlewski, L., et al., *(2004)* J Mol Biol, 2004. **336**(2): p. 307-11. [9] Alcocer, M.J., et al. (2004) J Mol Biol. **343**(3): p. 759-69. [10] Bacarese-Hamilton, T., et al., *(2004)* J Appl Microbiol. **96**(1): p. 10-7. [11] Belov, L., et al., *(2001)* Cancer Res. **61**(11): p. 4483-9. [12] Belov, L., et al., *(2003)* Proteomics. **3**(11): p. 2147-54. [13] De Ceuninck, F., L. Dassencourt, and P. Anract, *(2004)* Biochem Biophys Res Commun. **323**(3): p. 960-9. [14] Haab, B.B., *(2003).* Proteomics. **3**(11): p. 2116-22. [15] Miller, J.C., et al., *(2003).* Proteomics. **3**(1): p. 56-63. [16] Bacarese-Hamilton, T., et al., *(2004).* Methods Mol Biol. **264**: p. 271-83. [17] Klein, H.P., *(1974).* Orig Life. **5**(3): p. 431-41. [18] Klein, H.P., *(1979)* J Mol Evol. **14**(1-3): p. 161-5. [19] Klein, H.P., *(1978)* Orig Life. **9**(2): p. 157-60. [20] Levin, G.V. and P.A. Straat, *(1979).* J Mol Evol. **14**(1-3): p. 167-83. [21] Levin, G.V. and P.A. Straat, *(1977)* Biosystems. **9**(2-3): p. 165-74. [22] Yen, A.S., et al., *(2000)* Science. **289**(5486): p. 1909-12.