A PROTOTYPE LIFE DETECTION CHIP J. G. Maule and A. Steele, Geophysical Laboratory, Carnegie Institution of Washington, 5251 Broad Branch Road NW, Washington DC 20015. j.maule@gl.ciw.edu

Introduction: NASA's goal of "finding life beyond" will be addressed partly by *in situ* analysis of planetary rocks/regolith/soil. The scientific instrumentation on lander spacecraft should be lightweight, compact, stable in the space environment and be able to detect a diverse set of biological molecules, all of which may be present at very low concentrations.

We have developed prototype life-detection "chips", also known as antibody microarrays, consisting of several different antibodies[1] printed onto a glass surface. Each "chip" covers an area of 3mm^2, weighing about 10mg, stable over time. Chips are capable of detecting the following 10 biological molecules:

Target	Cell location	Abundance
DNA	Cell/nucleus	All organisms
L-glutamate	Cytosol	All organisms
PAH*	Memb. deriv.	All organisms
Chaperonin 60	Cytosol	All bacteria
β-galactosidase	Cytosol	All bacteria
Peptidoglycan	Membrane	G+ bacteria
Staphylococcus		Species specific
Lipopolysaccharide	Membrane	G- bacteria
Escherichia coli		Species specific
Mycoplasma	Cell	Bacteria/human respiratory tract

*Can also be generated non-biologically

Methods: Chips were generated following adaptations of previously described methods for high-throughput arrays [1]. A single microarray consisted of a 15 × 12 grid of spots, each 200μm in diameter, printed in quintuplicate using a robotic SpotBot arrayer (ArrayIt, Sunnyvale, CA). Each group of five spots contained a given concentration of a single antibody. Several different antibody concentrations were used (from 1000ng to 4ng per spot). Glass slides were pre-coated with a 15μm nitrocellulose layer (Schleicher and Schuell, Germany) that improved antibody binding and stability. At this stage, the 15 × 12 microarray was ready to receive a hydrated sample and could maintain function for at least several weeks. It is proposed that the microarray be sent to Mars in this configuration and incubated with hydrated regolith following landing. The surface of the microarray was then incubated with a hydrated sample of antigen for 1 hour at room temperature. The sample was then washed off with phosphate buffered saline (PBS) solution. The microarray incubated with Alexafluor-labeled antibody is then added to the chip, to complete a "sandwich assay". All these incubation steps have been performed successfully in Martian gravity [2]. Fluorescence was detected at 532nm and 635nm using an Axon 4000B scanner.

Results:

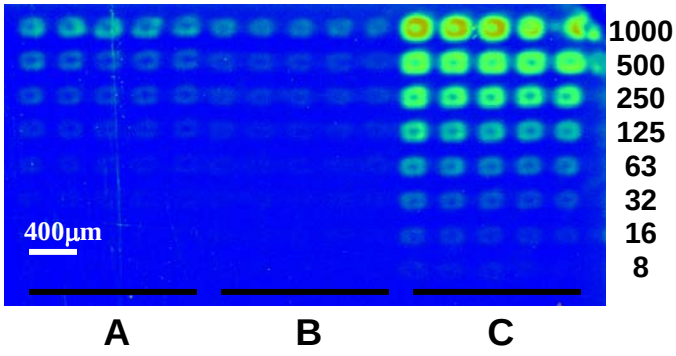

Figure 1: Single antibody microarray or "chip", scanned at 635nm.
A, Columns 1-5 printed with anti-LPS antibody. **B**, Columns 6-10, anti-PAH antibody. **C**, Anti-groEL antibody. Right-hand column shows concentration of antibody per spot. Sample solution tested contained groEL at sub-μM concentrations

Discussion:

The target molecules described here were chosen for life-detection purposes. We suggest that such a life-detection chip should have several layers of detection: **Broad** (e.g. DNA, all organisms), **Intermediate** (e.g. Chaperonin 60, most bacteria), **Group** (e.g. Peptidoglycan, gram-positive bacteria) and **Species** (e.g. *S. aureus*, a gram-positive species). For a Mars mission, it is essential that a test be performed in Earth-Mars transit as a control for contamination.

References:

[1] Macbeath G. and Schreiber, S.L. (2000) Science. 2000 Sep 8; 289(5485):1760.

[2] Maule J., Steele A. et al (2004) J. Gravit. Physiol. In press

[1] Antibodies (also known as immunoglobulins) are a group of proteins, produced by a white blood cell subset (B-lymphocytes) in most animals. They adhere strongly and highly specifically to a single molecular epitope (termed the "antigen") that is "foreign" to the host immune system. A wide range of mammalian (mouse, rabbit, goat) and avian antibodies can be purchased commercially, and are used widely in molecular biology research for the specific detection of molecular markers.