Mars Immunoassay Life Detection Instrument for Astrobiology (MILDI)
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The direct detection of organic biomarkers for living or fossil microbes on Mars by an in-situ instrument is a worthy goal for future lander missions. We have proposed an instrument based on immunological reactions to specific antibodies to cause activation of fluorescent stains. Antibodies are raised or acquired to a variety of general and specific substances that might be in Mars soil. These antibodies are then combined with various fluorescent stains and applied to micron sized numbered spots on a small (2-3cm) test plate where they become firmly attached after freeze drying. Using technology that has been developed for gene mining in DNA technology up to 10,000 tests per square inch can now be applied to a test plate [1,2]. On Mars or the planet / moon of interest, a sample of soil from a trench or drill core is extracted with water and/or an organic solvent that is then applied to the test plate. Any substance, which has an antibody on the test plate, will react with its antibody and activate its fluorescent stain. A small UV light source will illuminate the test plate, which is observed with a small CCD camera. The numbered spots that fluoresce indicate the presence of the tested-for substance, and the intensity indicates relative amounts. Furthermore with up to a thousand test plates available false positives and several variations of antibody can also be screened for. The entire instrument can be quite small and light, on the order of 10 cm in each dimension. A possible choice for light source may be small UV lasers at several wavelengths. Some of the wells or spots can contain simply standard fluorescent stains used to detect live cells, dead cells, DNA, etc. The stains in these spots may be directly activated, with no antibodies being necessary.

The proposed system will look for three classes of biomarkers: those from extant life, such as DNA, those from extinct life such as hopanes, and those from organic compounds not necessarily associated with life such as PAHs, rocket exhaust contamination and other a/pre-biotic chemicals. Both monoclonal and polyclonal antibodies can be used. Monoclonal antibodies react with a very specific compound, but polyclonal antibodies may react to any of a whole family of compounds. Furthermore the technique of phage display to raise antibodies against classically non antigenic molecules are also being considered [3]. Examples of potential biomarkers for which antibodies may / have been produced:

1. DNA, RNA and individual nucleotides including novel nucleotides used by the archaeabacteria.
2. ATP and ATP reductase.
3. Cyclic adenosine monophosphate.
4. Hopanes and other steroid-based membrane components which are known to survive for up to 2.5 billion years on Earth as specific biomarkers [4].
5. Lipopolysaccharides, probably of a cross section of species.
7. Porphyrins, including cytochromes, chlorophyll a, bacteriochlorophyll and the Ni and VO replaced porphyrin biomarkers found in oils.
8. Teichoic acids and other cell wall components.
9. Specific amino acid or peptide sequences and individual amino acids.
10. Flavin adenine dinucleotide (FAD) and nicotinamide adenine dinucleotide (NAD).
11. Antarctic cryptoendolithic biomarkers, specifically the specialized cryoprotectants and CV protectants such as Scytanemin.
12. RUBISCO.
13. Hydrogenase.
14. Nitrate reductase / Nitrogenase. Nitrogen metabolism may be the key to finding life, as all meteoritic carbon contains significantly lower levels of Nitrogen than earth soils. Therefore novel methods of Nitrogen fixation / utilisation must be considered a priority.
15. Specific PAHs, amino acids and nucleotide bases.
16. Environmental pollutants such as petroleum based contaminants (already available), plastics and potentially rocket exhaust residues.
17. Made on earth stamp. To nullify the possibility of the detection of terrestrial microbial contamination carried on the instrument, we propose to look for generic antibodies to terrestrial microbial cell wall components (several are currently available). Before launch these are applied to the instrument to label any possible contaminating organism with a ‘made on earth’ stamp. Antibodies against this stamp in the test wells will therefore show a positive reaction upon sampling terrestrial contamination.

This list is not exhaustive but serves to illustrate the possibilities and the range of antibodies currently being used in the medical, microbiological and environmental
pollution fields. Not only would such a test be able to indicate if traces of viable or non-viable life are present, if that life were viable, this list would enable biologists to determine what its composition was and even something about the metabolism of the organisms.

One aspect of this proposed experiment is that it must be extensively tested on a variety of terrestrial materials including soils to determine its detection limits, its propensity for false positives, and its ability to discriminate among related compounds. After successful application to both NASA and ESA for the now defunct 2005 mission work is currently underway to choose and evaluate a set of reasonable antibodies and currently we have a list of approximately 60 that we have access to or are immediately commercially available. Extensive laboratory testing of these antibodies will be achieved using the protein chip manufacturing and reading instrumentation at the Carnegie Institute of Washington. This instrument is capable of making protein/antibody arrays on a much bigger scale than is desirable for a flight instrument but it illustrates that the technology is currently available and needs only applying to the specific problem of robotic life detection. The mechanical design of the instrument is underway at Oceaneering Space systems in Houston.

Several key issues must be and are being addressed to ensure success of the concept, these include:
1. Number of steps from initial inoculation to produce a coherent fluorescent signal.
2. Issue of quenching and false positive screening.
3. Tailoring of suitable extraction methods.
5. Survival of reagents in various space simulate conditions.

The unique thing about these problems is for the first time the major hurdles to overcome in a flight instrument have a primarily biotechnological nature. A true extension of Astrobiology and the rationale for Astrobiology missions.

A major objective is to keep the instrument small and simple and to refine sample handling and extraction techniques as well as ascertaining the detection sensitivity of fluorescent and other detection mechanisms. An important feature of the instrument is its potential for multiple missions. Based on the results of the first mission, the mix of antibodies can be modified, and the instrument can be flown again tailored to zero in on some additional likely compounds. Although this instrument must initially be tailored to a terrestrial organism baseline, so must all initial rationales for life detection. It is only by the application of such specific techniques that we will be able to assess results and modify the search parameters for potentially more exotic metabolisms and biomarkers. Techniques such as phage display may then come into there own as they can be used to screen for a range of probable mutations / differences in a specific antigen.

Once manufactured the antibodies within these chips can be tailored to a range of applications including automated environmental monitoring for manned missions and sample curation issues, medical screening of Astronauts as well as hand held laboratory instruments for manned missions. Currently our target is to

A further application of immunological based detection is Chemical Force Microscopy (CFM). In this technique the tip of an Atomic Force Microscope (AFM) is modified using an antibody. As the tip is scanned across the surface of the sample the force at which the tip interacts with the surface can be measured. As the antibody comes into contact with the antigen on the surface the force of the tip/surface interaction (tip retraction) increases. This can be measured and subsequently imaged across the sample surface in 3-D with nanometer resolution [5]. Imaging of Martian meteorites with AFM has already been shown to be viable and useful [6]. This technique will be used to verify MILDI results and to act as quality control in the final antibody chip manufacturing process.

References
[1] Lueking et al. (1999) Anal Biochem 207; 103

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