A MULTIPLEX COMPETITIVE IMMUNOASSAY FOR THE DETECTION OF ORGANIC COMPOUNDS ON MARS. M. Moreno-Paz[1], A. Gómez-Cifuentes[1], O. Hosftetter[2], A. Maqueira[3], S. Morais[3], D. Knopp[4], and V. Parro[1]. [1]Centro de Astrobiología (CAB, INTA-CSIC), Madrid, Spain, parrogv@cab.inta-csic.es; [2]Northern Illynois University DeKalb, IL, USA; [3]Universidad Politécnica de Valencia, Spain; [4]Technische Universität München, Germany.

Introduction: The instrumentation used so far for detecting organic matter on Mars have been based on GC/MS analysis of volatile compounds obtained after heating at high temperatures (100-1000ºC). Since the discovery of perchlorate on Mars and the destructive effect on organic matter at high tempertures, new and mild methods for the detection of intact organic molecules are needed. We propose the SOLID (Signs of Life Detector) instrument concept based on wet and low temperature analysis by using antibody microarray technology [1]. SOLID has two functional units, the Sample Preparation Unit (SPU) and the Sample Analysis Unit (SAU). The SPU extracts the organic matter into a liquid buffer by ultrasonication and the SAU analyses this extract by higly specific immunological assays. In the case of small size molecules a Competitive or Inhibitory Immunoassay (CI) has to be developed. Among the potentical martian molecular targets are those supplied by meteorites (D and L amino acids, PAHs) or by a hypothetical martian biota (aromatic amino acids, esteroid and triterpene molecules, small peptides).

Objective: To develop and demonstrate a multiplex competitive/Inhibitory immunoassay for simultaneous detection or organic molecules relevant for Mars exploration.

Methods: We developed a multiplex competitive immunoassay for simultaneous detection of small and relevant analytes for planetary exploration: generic D-amino acid group (NH2-CHR-COOH), generic L-amino acids [2], and polyaromatic hydrocarbons (PAHs) as the benzo[a]pyrene [3]. Other molecules highly relevant for environmental monitoring on Earth and structurally relevant for organic detection on Mars have been included: atrazine, finasteride, phthalylsulfathiazole, pentachlorophenol, sulfamethazine [4]. Each compound was conjugated to a protein and printed on epoxy-activated microscope glass slides.

Competitive Inhibitory Immunoassay (CI). Reactive spots in a microarray are coated with conjugate molecules—large molecules carrying a small target compound. For analysis, first the microarray is flooded with fluorescent Ab's that bind specifically to conjugates. An image of the microarray is obtained after exciting fluorescence with a laser. This image represents 100% of positive Ab-Conjugate coupling and is used as a baseline. Next, a liquid extract from the SOLID-SPU is mixed with fluorescent Ab's in the microarray. Martian organic analytes compete with conjugates for the fluorescent Ab's. After up several minutes of incubation a second fluorescence image of the microarray is obtained. If a martian-analyte is present, the fluorescence intensity of the corresponding spots diminishes proportionally. The higher the concentration of martian analyte, the lower the signal intensity from the spot.

Results: We tested one by one the different pairs analyte-Ab by CI and determined the specificity and the limit of detection (which ranged from few ppb to ppm). Further, we determined their performance by multiplex assay using mixtures of all the antibodies and detecting single analytes. Finally, natural soil samples from the Antarctic Dry Valleys were spiked with the analytes, extracted and analyzed following SOLID procedures. The results (Fig. 1) showed a clear (>80%) and specific loss of fluorescent signal for each compound and a complete inhibition when using higher concentrations.

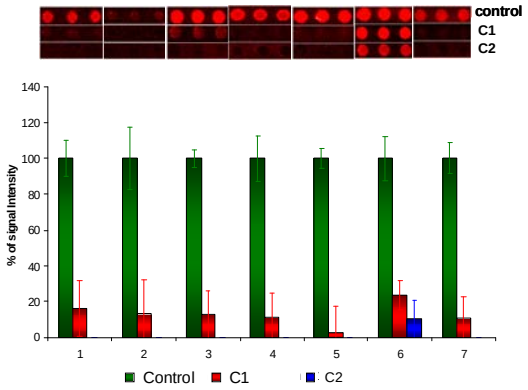

Figure 1. Multiplex competitive fluorescence inmunoassay. Mixtures of seven analytes were tested at two concentrations (C1/C2): 1) 0.1/10 ppm of Atrazine; 2) 0.01/100 ppb Finasteride; 3) 1/100 ppm Phthalylsulfathiazole; 4) 0.1/1ppm Pentachlorophenol; 5) 0.1/10 ppm Sulfamethazine; 6) 20/100ppm D-Phe; 7) 1/10 ppm L-Phe. *Green bars,* 100% fluorescent signal for each compound.

References: [1] Parro et al. (2011) *Astrobiology* 11: 15-28. [2] T. Kassa et al. (2011) *Analyst* 136: 1113-15. [3] X.Y.Z. Karsunke et al. (2011) *J. Immunol. Methods* 371:81–90. [4] E. M. Brun et al. (2010). *Anal. Chim. Acta* 671: 70-79

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