THE SOLID3 ("SIGNS OF LIFE DETECTOR") INSTRUMENT: AN ANTIBODY MICROARRAY-BASED BIOSENSOR FOR PLANETARY EXPLORATION. V. Parro[1], L. A. Rivas[1], E. Sebastián[1], Y. Blanco[1], J. A. Rodríguez-Manfredi[1], G. de Diego-Castilla[1], M. Moreno-Paz[1], M. García-Villadangos[1], C. Compostizo[2], P. L. Herrero[2], A. García-Marín[2], J. Martín-Soler[1], J. Romeral[1], P. Cruz-Gil[1], O. Prieto-Ballesteros[1], and J. Gómez-Elvira[1] , [1]Centro de Astrobiología (INTA-CSIC), Carretera de Ajalvir km 4, Torrejón de Ardoz, 28850, Madrid, Spain, [2]SENER Ingeniería y Sistemas S. A. Avda. Zugazarte 56, 48930, Las Arenas, Vizcaya, Spain

Introduction: To date, the technology used to detect organic molecules on Mars has been based on the detection of volatile compounds by gas chromatography–mass spectrometry after a ramp of heating (up to 750 ºC) or a direct pyrolytic process (1000–1100ºC). One of the most relevant discoveries from the Phoenix lander was the detection of a relatively high concentration of perchlorate (ClO_4^-) at the landing site, around 2.4 mM [1]. It is known that the presence of perchlorate at high temperature destroys organic matter, and this can be a reasonable explanation for the negative results obtained in the Viking and Phoenix missions, as has been suggested [2]. Consequently, to detect organic compounds and biomolecules, it is necessary to develop new and robust technologies compatible with the martian soil chemistry and independent from the type and properties of the substrate. One of the most promising technologies is based on immunosensors. We have previously reported the Signs Of LIfe Detector (SOLID) concept [3, 4, 5] an antibody microarray-based biosensor for *in situ* analysis. A field prototype (SOLID2) was tested during the MARTE project, a NASA-CAB Mars drilling simulation experiment [5]. The last version, the SOLID3.0 [6] loaded with LDChip300 (an immuosensor with more than 300 antibodies) has been successfully tested recently in Atacama Desert [7] and Deception Island (Antarctica) [8].

LDChip, a life detector chip for planetary exploration. We hypothesize that microorganisms living under similar environmental conditions share similar molecular mechanisms to deal with such environments. These mechanisms will render common molecules that eventually could be good targets to search for life. Antibodies can be highly specific and capable of distinguishing between two enantiomers or show a relaxed specificity to recognize similar but not identical structures. We have focused on terrestrial analogues for Mars, and we have produced polyclonal antibodies against extracts from the acidic, iron-, and sulfur-rich environments, hydrothermal environments, permafrost, and so on. Samples were taken from water, sediments, mineral deposits (sulfate precipitates, jarosite, hematite, etc.), rocks, and the subsurface during drilling campaigns. At the same time, we are performing biochemical fractionation from pure bacterial cultures so that we can produce antibodies against different types of macromolecules (EPS, anionic polymers, cell wall components, etc). We have also produced antibodies against pure bacterial cultures (type collections) isolated from cold (Artic and Antarctic), hydrothermal, saline, dry environments, and so on [9, 10]. We currently have a collection of nearly 450 antibodies, including all mentioned categories.

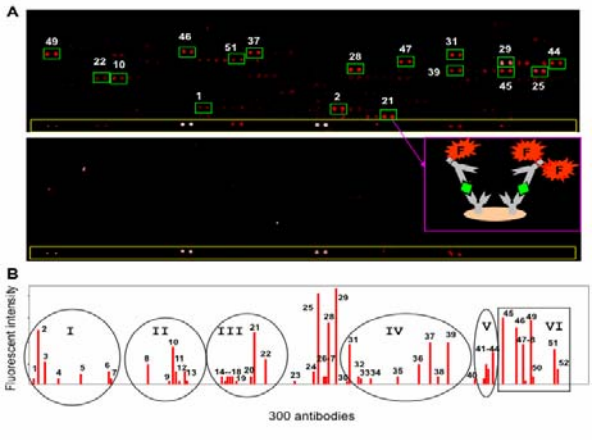

Fig. 1. (A) An LDChip300 image obtained from the analysis of a sample from 2 m depth of Atacama Desert subsurface showing fluorescent positive reactions. The cartoon shows how capturing antibodies (Y forms) bind biological polymers (rhombi) and how they are sandwiched by fluorescent (F) tracer antibodies. The image obtained with the negative control (bottom) was used as a baseline (blank) to calculate spot intensities in the test samples. Long yellow rectangles encompass a fluorescent spot gradient. (B) Immunogram showing the fluorescence intensity of the positive spots corresponding to antibodies against microbes and biological polymers. From ref. [7].

SOLID3, a lab-on-a chip based instrument ready for planetary exploration. SOLID is a lab-on-a-chip powerful analytical instrument based on antibody array technology, that can detect a broad range of molecular size compounds, from the amino acid size level to whole cells, with sensitivities at 1-2 ppb (ng ml^{-1}) for biomolecules and 10^4-10^5 cells ml^{-1} [6, 9]. The SOLID instrument is currently in TRL 5-6, although some of the internal elements are in TRL 9 stage (like CCD). Antibodies and fluorochromes have been tested under space conditions (high energy proton radiation, gamma

radiation, suddent temperature shifts, long time storage, etc) and showed no significant loss of performance when treated accordingly [11]. Additionally, we proved that more than ten times perchlorate concentration did not affect the SOLID immunoassay [6]. Therefore, some minor changes in main components shall need to be accomplished, in order to be tailored to a mission. SOLID can be autonomous from the thermal control point of view. Suitable heaters can be used to guarantee that fluids are in operative range (higher than -5 ºC). The instrument accepts both solid (dust or grinded rock or ice) and liquid samples. Limits on sample volume range from 0.5-1 cm^3, and up to 2.5 cm^3 (ml) at final processing volume (including the extraction buffer supplied by SOLID). For soil samples there is no special grain size requirements, but grains below 1 mm in diameter are desirable.

How SOLID work. SOLID is divided into two main units: the sample preparation unit (SPU), and the sample analysis unit (SAU), bearing the antibody microarray biosensor (the Life Detector Chip or LDChip) for the immunological assays (Fig. 2). For each assay, up to 1 g of soil, ground rock, powder or ice is loaded into the extraction cell through the loading port [6]. Then, a sonicator horn moves forwards so that it displaces a close membrane-ring confining the sample in a hermetic chamber. The extraction buffer (2 ml of TBST-RR) is injected from the opposite direction of the exit flow, and the ultrasonicator is powered on to perform 3 to 10 cycles of ultrasonication (1 min each). The sonicator horn and membrane-ring acting as a piston push forward the sample, which is filtrated through 10-15 micron pore size filter. The filtrate can then be injected to the microarray chamber of the SAU, where it will be directly analyzed. After the first sample has been analyzed, the SPU pipes and valves are rinsed to avoid cross-contamination between samples and obstruction of the valves by particulate matter. The SAU consists of one analysis module, equipped with 5 flow cells, which allocate one antibody microarrays each, as well as the motor, pumps, fluidics, valves, etc., necessary to perform the analysis. The analyte containing solution from the SPU is incubated with the antibody microarray in the flow cell to perform a sandwich microarray immunoassay, or a competitive microarray immunoassay. In both cases, after the assay is completed, the Signal Readout Module subsystem is activated: a laser beam enters by the front edge of the microarray support and is transmitted by total internal reflexion using the same support as a waveguide. The light excites the fluorochromes on the spots and the fluorescence signal is captured through a micro-lens array by a CCD device. We designed a specific optical package in order to capture a relatively big surface area with the minimal mass allowable. In addition, the compact optical package also allows a compact and light SAU (around 1 kg).

Fig. 2. The SOLID3 instrument in the field, showing its two functional units: the Sample Preparation Unit (SPU) (right) and the Sample Analysis Unit (SAU))left. From ref. [7].

SOLID and LDChip for human protection in planetary exploration. Human exploration of Mars would require safety procedures. In addition to the *in situ* life detection capabilities, SOLID-LDChip can contribute to ascertain the presence or absence of potential pathogens on Mars or in an hypothetical habitable module. Specific LDChips can be designed for the detection of human, animal or plant pathogens, or even toxins.

References: [1] Hecht M.H. *et al.* (2009) *Science* 325, 64–67. [2] Navarro-González R. *et al.*, (2010) JGR 115, doi:10.1029/2010JE003599. [3] Parro, V. *et al.*, (2005) *Planet. Space Sci.* 53, 729-737. [4] Parro, V. *et al.*, (2008) *Space Sci. Rev.* 135, 293–311. [5] Parro, V. *et al.*, (2008) *Astrobiology* 8, 987-99. [6] Parro, V. *et al.* (2011) *Astrobiology* 11, 15-28. [7] Parro, V. *et al.* (2011) *Astrobiology* 11, 969-96. [8] Blanco Y. *et al.*, (2012) *Environ. Microbiol.* (in press). [9] Rivas, L.A. *et al.* (2008) *Anal. Chem.* 80, 7970–7979. [10] Rivas L.A. *et al.*, (2011) *Environ. Microbiol* 13, 1421-1432. [11] De Diego-Castilla et al., (2011) *Astrobiology* 11, 759-73.