

SIGNS OF LIFE DETECTOR (SOLID): SEARCHING FOR EVIDENCE OF PAST LIFE ON MARS. V. Parro¹, C. Stoker², A.F. Davila³, R. Quinn⁴. ¹Centro de Astrobiología (parrogv@inta.es), ²NASA Ames Research Center (carol.stoker@nasa.gov), ³Carl Sagan Center at the SETI Institute (adavila@seti.org), ⁴Carl Sagan Center at the SETI Institute (richard.c.quinn@nasa.gov).

Introduction: The case for life on Mars grows stronger. Investigations at Gale Crater by *Curiosity* have revealed fine-grained sedimentary rocks inferred to represent an ancient lake environment suited to support life [1]. In addition, *Curiosity* tentatively found a heterogeneous distribution of organic C within these sediments [2], consistent with the detection of native organic C in Mars meteorites [3]. However, combustion of organics due to the presence of perchlorate during thermal analysis have prevented the identification of parent organic C compounds. Now we need to understand the nature and distribution of organic C; to constrain the preservation potential of biosignatures; and to determine if evidence of life is preserved in the rock record.

The SOLID Instrument: The Signs of Life Detector (SOLID) is a mature instrument with state of the art liquid extraction and lab-on-a chip immunoassay technology to detect and characterize organic C on Mars (Fig. 1). The instrument has a lineage of technology development since 2000 [4-6]. The SOLID instrument concept is based on the automation of fluorescent microarray immunoassays. The instrument is divided into two physically separated units: the Sample Preparation Unit (SPU) for extraction of organic molecules from powdered rock using liquid sonication; and the Sample Analysis Unit (SAU) for production of fluorescence

images with the results of the immunoassay.

Conceptually, immunoassays mimic our immune system in that a fleet of antibodies (Ab) is designed to chemically capture desired organic C compounds termed antigens (Ag) in solution. Antibodies possess high specificity and affinity for their respective Ag, making immunoassays both reliable and sensitive, with confirmed detection limits of a few ppb for specific compounds. For analysis, the liquid extract obtained in the SPU is exposed to a fleet of Ab's designed to anchor to a specific region of the target organic compound. Special labeled Ab's containing a fluorescent tag that can be excited with a laser are used to reveal the presence of the Ag in the sample. State-of-the-art technology allows immunoassays-on-a-chip, whereby Ag identification occurs in a microchip, with minimal mass, volume and power requirement. SOLID can perform two assays, depending on the type of targeted organic compound: *Competitive Inhibitory Immunoassay* for relatively simple and small organic compounds such as amino acids, and *Sandwich Immunoassay* for larger and more complex organic compounds such as peptides or nucleic acids (Table 1).

SOLID performance features: SOLID extracts organic C compounds from rock samples using liquid sonication, circumventing the problem of pyrolysis extraction in the presence of thermally unstable per-

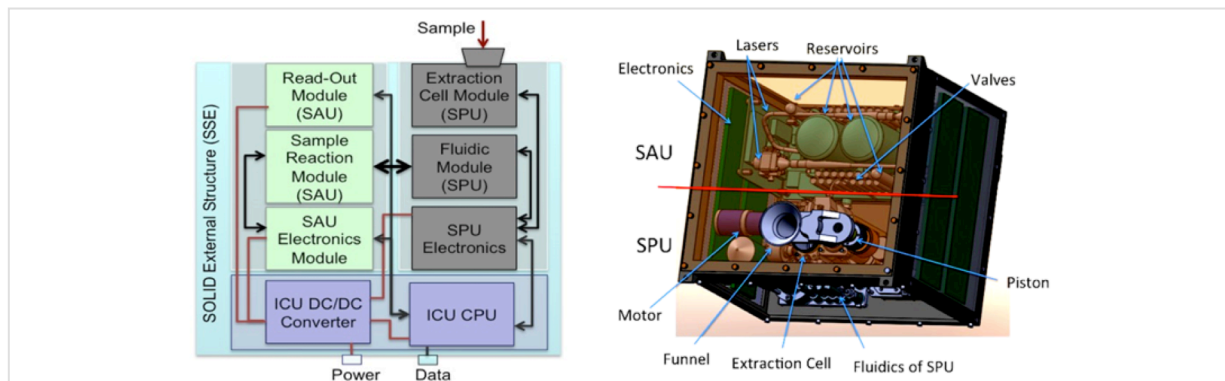


Figure 1 (Left) SOLID Instrument Block Diagram showing 3 main modules. Power is provided by ICU, which collects internal sensor and housekeeping information and generates internal commands. ICU is the single power and data interface with the rover. **(Right) The packaged SOLID instrument** has an external structure (SES) attached to the RAMP that isolates all internal units from the interior of the rover body, assuring that the instrument can't contaminate the rover body. Its sample delivery funnel is closed by a lid when not delivering sample. The SAU is attached to the lower wall of SES and the SPU to both upper and lower walls. All the electronics are attached to the lateral walls.

chlorate. The instrument uses buffer solutions to compensate for strong pH changes during extraction, and can operate at above typical Martian salt concentrations—liquid extraction and analysis is compatible with up to 20 times the average perchlorate concentration on Mars [4]. Maximum extraction temperature is c.a. 80°C, but samples are analyzed at normal temperature to avoid differential precipitation of soluble phases. SOLID's core sensor is the BioMarker Chip (BMC) a microarray with 1008 Ag-Ab reaction spots at pre-assigned and known locations. Each spot contains millions of molecules of capturing Abs (for SI) or Ag-conjugates (for CI). SOLID is designed to conduct up to 30 sample analyses. Each analysis requires 2 grams of material. Tests using Martian soil simulant JSC Mars-1 have shown extraction efficiencies of 50% for highly hydrophobic compounds and more than 90% for water soluble ones.

To address possible contamination issues SOLID implements three strategies: 1) The sampling mechanism delivers samples from surface materials known to be devoid of organic carbon. Any contaminants from Earth in the sampling chain or within the instrument will be diluted after repeated sampling; 2) A control analysis is run prior to each sample analysis utilizing only the instruments internal buffers and antibody solutions. Possible contaminants from Earth are revealed in this control run, and subtracted from further analyses; 3) To distinguish fluorescence signal from terrestrial forward contamination, several antibody spots in the BMC are dedicated to detect microbial strains known to be present in clean rooms and resistant to thermal treatment.

SOLID performance: The lower limit of detection (LOD) for organic C compounds ranges from 1 to 500 ppb levels (ng ml^{-1}) in the liquid extract [4]. The actual sensitivity is a function of the quality of each antibody, the extraction efficiency, and the initial volume of sample delivered. SOLID can differentiate between organic compounds ranging from simple amino acids to complex polymer and hydrocarbons, and detect as little as 10^4 cells/gr to assess forward contamination.

SOLID's role in Mars Exploration: SOLID investigations can link directly to, and logically follow, the unprecedented successes achieved by the NASA Mars Exploration Program over the last two decades, best exemplified by the discovery of ancient habitable environments [e.g., 1,7] and of possible organic C [2]. The future of Mars exploration, as recommended in the NRC Decadal Survey for Planetary Science, is sample return [8]. SOLID investigations can play a key role in the Mars2020 Mission and future sample return, by searching for high priority samples identified by their organic C content and potential for preserved evidence of past life. SOLID also assesses possible forward contamination of Mars by targeting biosignatures of terrestrial life possibly transported to the planet, a key knowledge gap in Planetary Protection.

References: [1] Grotzinger et al. (2013) *Science* DOI:10.1126/science.1242777. [2] Ming et al. (2013) *Science* DOI:10.1126/science.1245267. [3] Steele et al. (2012) *Science* 337, 212 [4] Parro et al. (2011) *Astrobiology* 11, 969. [5] Fernández-Remolar et al. *JGR* 118, 922. [6] Blanco et al. *Environ. Microbiol.* 14, 2495. Squyres et al. (2004) *Science* 306, 1709. Planetary Decadal Survey (2012) *NRC*.

Table 1. Molecules that can be detected with SOLID and the implications of their detection on Mars

Simple abiotic organics (Category 1)	Implications of detection
Meteoritic amino acids (Alpha-aa, D-aa, Ala, Asp, Glu, Val) PAHs and photoproducts (Mellitic, Benzoic Naphthalene, Pyrene, Anthracene, Quinoids) Meteoritic nucleobases (A, G, C, U, Xa)	Elevated potential for biosignatures. Sample should be selected for return. Meteorites deliver organics to Mars Building blocks of life are present
Possible organic biomarkers (Category 2)	
Aromatic amino acids (Phe, Tyr, Try) Nucleotide & derivatives (AMP; GMP; C-GMP)	The potential for biosignatures is high. Sample should be selected for return. Prebiotic or biotic chemistry on Mars
Definitive organic biomarkers (Category 3)	
Hydrocarbons (n-Alkane, Kerogen, Alkyldibenzothiphenene, Isoprenoid, Carotenoid, Alkylphenanthrene) Lipids (Hopanes, Steranes, Lycopanes, Carotanes) Polymers (Peptides, Nucleic Acids, Polysaccharides) Heterocycles (Porphyrin ring)	Definitive biosignatures Life once existed on Mars Sample should be selected for return. Planetary Protection concerns
Earth Organisms (<i>Bacillus</i> , <i>Streptomyces</i> , <i>Pseudomonas</i> , <i>E. coli</i> , <i>Clostridium Propionibacterium</i>)	There has been forward contamination with Earth microorganisms