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Report of the workshop for life detection in samples from Mars



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ABSTRACT

The question of whether there is or was life on Mars has been one of the most pivotal since Schiaparelli's telescopic observations of the red planet. With the advent of the space age, this question can be addressed directly by exploring the surface of Mars and by bringing samples to Earth for analysis. The latter, however, is not free of problems. Life can be found virtually everywhere on Earth. Hence the potential for contaminating the Mars samples and compromising their scientific integrity is not negligible. Conversely, if life is present in samples from Mars, this may represent a potential source of extraterrestrial biological contamination for Earth. A range of measures and policies, collectively termed 'planetary protection', are employed to minimise risks and thereby prevent undesirable consequences for the terrestrial biosphere. This report documents discussions and conclusions from a workshop held in 2012, which followed a public conference focused on current capabilities for performing life-detection studies on Mars samples. The workshop focused on the evaluation of Mars samples that would maximise scientific productivity and inform decision making in the context of planetary protection. Workshop participants developed a strong consensus that the same measurements could be employed to effectively

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inform both science and planetary protection, when applied in the context of two competing hypotheses: 1) that there is **no** detectable life in the samples; or 2) that there is **martian** life in the samples. Participants then outlined a sequence for sample processing and defined analytical methods that would test these hypotheses. They also identified critical developments to enable the analysis of samples from Mars.

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1. Introduction

Mars Sample Return (MSR) has been proposed to answer certain compelling scientific questions by means of collecting a set of carefully selected, diverse samples on Mars for subsequent analysis in terrestrial laboratories. Sample selection, storage and transport would need to be conducted in a way that avoids compromising their scientific integrity (iMars, 2008; MEPAG ND-SAG, 2008; MEPAG E2E-ISAG, 2012). An MSR program would also have to meet stringent planetary protection constraints in line with international regulations stipulated by the Committee on Space Research (COSPAR) and the United Nations Treaty on Principles Governing the Activities of States in the Exploration and Use of Outer Space, including the Moon and other Celestial Bodies. These planetary protection constraints have been put in place to avoid adverse changes in the environment of the Earth resulting from the introduction of extraterrestrial matter (NRC, 2009; ESF, 2012). In practical terms, this means that the samples from Mars must initially be opened and examined under full biological containment to assess if they contain signs of life. Whether subsequently the samples are kept in containment, released from containment after sterilisation, or curated without any containment constraints will depend on the results of a comprehensive sequence of tests. The first step to develop a protocol for these tests started in 2001 through a series of workshops, resulting in a Planetary Protection Draft Test Protocol (Rummel et al., 2002). One of the recommendations of this early work was to periodically review and update the Planetary Protection Draft Test Protocol to incorporate new scientific findings about Mars, and technical advances in the analytical capabilities and methodologies to detect the signs of life.

The National Aeronautics and Space Administration (NASA) and the European Space Agency (ESA), in coordination with COSPAR, work together to update elements of the Planetary Protection Draft Test Protocol. These activities include a general Life-Detection Science Conference to discuss, to determine and to assess the latest concepts and methods to search for life on Mars and a more focused Life-Detection Workshop to identify relevant elements for a Planetary Protection Test Protocol.

The main conclusions of the Life-Detection Science Conference were used as starting points for the Life-Detection Workshop (Allwood et al., 2013):

- Employing a hypothesis-driven approach in the development of life-detection investigation strategies and measurements for

science (null hypothesis = there is no life in the sample) and planetary protection (positive hypothesis = there is life in the sample) provides a sound framework.

- Disproving either the positive or null hypothesis can only be accomplished by collecting a sufficient amount of statistically significant data.
- To avoid the ambiguity of the term “biosignature”, it is proposed to use the terms “signs of life” or “evidence of life” referring to a feature or set of phenomena judged to be of biological origin.
- Evidence for life lies not in a single, “smoking gun” observation, but rather in a suite of observations spanning samples and different contexts.

For the purposes of the Planetary Protection Test Protocol, any indications of viable, dormant, or recently deceased life forms, as well as fossils and the trace evidence of life processes, are all considered to be signs of life. The term “sample container” is used in this report to describe the physical containers holding the individual Mars samples, and not the sample containment that isolates the samples from the terrestrial environment. The sample containment is provided by a separate system around the sample container (e.g., flight containment system or containment elements in a terrestrial containment facility).

2. Workshop organisation

An organisation committee was established under the leadership of the NASA and ESA Planetary Protection Officers to prepare the workshop agenda and to identify key individuals for participation. The workshop took place at the Scripps Institution of Oceanography, La Jolla, California, from 15 to 17 February 2012. A Life-Detection Science Conference, held at the same location over the three days preceding the workshop, provided valuable inputs. The workshop started with a recapitulation of the Life-Detection Science Conference, and continued with short talks given by the 33 participants to establish a common basis for the subsequent discussions. The participants were then split in three topical groups according to their expertise: 1) deep ocean/subsurface, 2) glacier/permafrost, and 3) hyperarid/hypersaline. These topical groups were tasked with answering the following question: How would you detect signs of life in an extraterrestrial sample? The notes of the three groups were presented and discussed in a plenary before the participants were re-organised into two groups, representing a mixed set of expertise in both groups. The two groups were requested to develop a life-detection protocol specifically for samples from Mars. Notes from the two groups were presented and discussed in a plenary session, in addition to a dedicated presentation on the statistical approaches that could be employed for establishing confidence in claims that life was detected. The current report summarises the workshop discussions and presents the consolidated conclusions.

3. Objectives for the workshop

The main objectives for the workshop were to:

1. Review the Planetary Protection Draft Test Protocol (Rummel et al., 2002), taking into account the latest information about

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Table 1Proposed sequence for sample analysis. For the specific types of analysis see [Table 2](#).

Sequence for sample analysis	Sample condition	General type of analysis
I	Sample acquisition on Mars	Remote and <i>in-situ</i> analysis on Mars to characterise the sample type and the geological context
II	Any solid sample material on the outside of the sample containers	Solid sample analysis; full sequence (non-destructive & non-invasive, non-destructive & minimal invasive, and destructive)
III	Head space gas	Gas sample analysis; full sequence
IV	Solid samples in containers	Solid sample analysis; non-destructive & non-invasive
V	Solid samples removed from containers	Solid sample analysis; non-destructive & minimal invasive
VI	Fluid inclusions from solid samples removed from containers	Liquid sample analysis; full sequence
VII	Solid sample removed from containers	Solid sample analysis; non-destructive & minimal invasive, destructive

Table 2

Examples for types of sample analysis with a focus on life-detection. The detailed processing of the solid samples would depend on their nature, e.g., rocks, regolith, sand, and requires proper contamination control. FTICR-MS: Fourier Transform Ion Cyclotron Resonance Mass Spectrometry; GC-IRMS: Gas Chromatography Isotope Ratio Mass Spectrometry; GC-MS: Gas Chromatography Mass Spectrometry; IR: Infrared; LC-MS: Liquid Chromatography Mass Spectrometry; SEM: Scanning Electron Microscopy; TEM: Transmission Electron Microscopy; TOF-SIMS: Time of Flight Secondary Ion Mass Spectrometry; UV: Ultraviolet; XANES: X-Ray Absorption Near Edge Spectroscopy; XRD: X-Ray Diffraction.

Invasiveness	Solid sample analysis	Gas sample analysis	Liquid sample analysis
Non-destructive & non-invasive	<ul style="list-style-type: none"> • 3D X-ray micro-tomography • Surface imaging and spectroscopy 	Not applicable	Not applicable
Non-destructive & minimal invasive (no specific sample preparation)	<ul style="list-style-type: none"> • Microscopy • Fluorescence • IR, visible, UV, deep UV spectroscopy • SEM 	<ul style="list-style-type: none"> • IR, visible, UV, deep UV spectroscopy 	<ul style="list-style-type: none"> • Microscopy • Fluorescence • IR, visible, UV, deep UV spectroscopy
Destructive (specific sample preparation)	<ul style="list-style-type: none"> • SEM, TEM, nano-X-ray-tomography • XRD, XANES • GC-MS, GC-IRMS, FTICR-MS, LC-MS, TOF-SIMS, Nano-SIMS • Target independent biopolymer sequencing 	<ul style="list-style-type: none"> • GC-MS, GC-IRMS, FTICR-MS, LC-MS 	<ul style="list-style-type: none"> • GC-MS, GC-IRMS, FTICR-MS, LC-MS, TOF-SIMS, Nano-SIMS, • Target independent biopolymer sequencing, flow cytometry

Mars and the discussions and conclusions of the Life-Detection Science Conference ([Allwood et al., 2013](#)).

2. Identify necessary research and technical developments to establish and execute a future Planetary Protection Test Protocol.
3. Identify issues that would result in requirements for the design of future sample return flight hardware, in particular hardware for sample acquisition, storage, and transportation.

It was not the objective of the workshop to update the current Planetary Protection Draft Test Protocol.

4. Proposed sequence of analysis for life-detection

An MSR program is scientifically most useful if it returns three distinct classes of samples: rocks, granular material (regolith and sand), and atmospheric samples ([MEPAG E2E-iSAG, 2012](#)). The different types of samples will likely require different processing strategies, extraction procedures, and analytical approaches. The analysis of martian samples must begin with the *in-situ* characterisation of the geological context during sample acquisition on Mars. Parameters like sample type and basic chemistry must be established in order to tailor the subsequent sample analysis on Earth and for the associated interpretation of the data. Samples could be soft sediments or well lithified, and may also contain fluid and gas inclusions. Primary phases may be highly ordered and crystalline, poorly ordered, or amorphous. The composition could be highly diverse including sulphates, chlorides, nitrates, (per)chlorates, bromides, iodates, bromates, organics, etc. All these factors need to be considered during sample acquisition, storage and analysis in order to preserve potential biological material.

The proposed overall sequence for sample analysis and for defining the type of analytical methods were derived by consensus of the workshop participants and are detailed in [Table 1](#) and [Table 2](#), respectively. The described analytical methods reflect the purpose of this workshop, i.e. to establish if life is present in the

samples, and are therefore part of a larger set of analytical methods that would be employed on the samples from Mars.

The first Mars samples to be analysed on Earth would be the martian material adhering to the outside of the individual sample containers following the solid sample analysis track ([Table 2](#)). This would be followed by extraction of gases in the head space of the sample containers and by processing them according to a gas sample analysis track ([Table 2](#)).

The next step in the sample analysis would include non-destructive and non-invasive measurements for characterising the samples while still within their individual sample containers. Three-dimensional (3D) X-ray micro-tomography of the individual samples would be of critical importance to plan subsequent handling, sub-sampling, and analysis. Such 3D imaging would provide a reference frame for each sample and information about the physical heterogeneity at micron-level resolution, including evidence for open fractures, veins, primary pore spaces, and the presence of mineralogical and/or lithological heterogeneities ([Friedrich and Rivers, 2013](#); [Needham et al., 2013](#); [Tsuchiyama et al., 2013](#)). Computerised tomography using a higher X-ray dose (e.g., synchrotron radiation) could be applied to determine compositional information, including elemental distribution and mineralogy. However, potential negative effects on sample integrity caused by considerably higher X-ray doses for compositional mapping need to be evaluated further. Surface imaging and spectroscopy carried out prior to extracting the samples from their individual canisters would be complementary to the X-ray tomography and would also be informative for subsequent sample processing.

Removing solid samples from their containers will most likely occur only after reviewing (at least) preliminary results from the analysis of the external martian material, head space gas, and 3D imaging of the samples. The individual solid samples from the sample containers would need to be visually inspected. They

might be intact cores or fragments of differing size and shape with certain levels of fines. Accessible surfaces of the removed samples, in particular freshly exposed surfaces, should be investigated first with non-destructive and minimally invasive methods such as passive and active spectroscopic methods and various imaging techniques (Table 2). Ideally, these methods would provide both macro- and microscale information on potential organics, mineralogy, elemental composition, morphology, and help to document any changes compared to the *in-situ* characterisation on Mars.

Further sub-sampling and sample processing for destructive analysis would be based on X-ray tomography (in particular for intact cores and larger fragments) and the previous non-destructive analyses. This destructive and invasive sample processing would be accomplished by analytical techniques that require sample preparation and/or extraction-based analysis to get a wide range of high-resolution morphological, molecular, chemical, and isotopic information (Table 2). The extraction-based methods, whether using heat (e.g., pyrolysis or laser desorption) or liquid extraction (e.g., solvent extraction at different temperatures), would need to be tailored to the specific chemistries of the samples to avoid the destruction of vital information and to ensure that a maximum amount of scientific information can be obtained.

A carefully tailored rehydration protocol is of particular importance for wet chemical analysis to ensure that molecular or cellular structures are not destroyed before they can be detected and analysed.

Appropriate means for monitoring terrestrial contamination levels (e.g., inert material samples, witness plates) during sample acquisition on Mars, storage, transfer from Mars to Earth and during handling on Earth are critical. Appropriate and well-characterised naturally occurring terrestrial analogues and synthetic (i.e. artificial) analogues should be used to test the entire analytical sequence, while being mindful of potential sample cross-contamination. In particular, synthetic analogues could be highly valuable during the processing of martian samples so as to be rapidly responsive to the data and support the interpretation of the data. Such synthetic analogues could mimic both the chemical and physical characteristic of the Mars samples before using specific analytical techniques and extraction procedures.

A key workshop conclusion is that the same measurements scientists would perform on the samples would effectively inform both science and planetary protection. The distinction between the science and planetary protection elements would mainly be in setting a clear decision making framework in line with the Earth safety aspect of planetary protection and relevant national and international regulations.

5. Conclusions

The participants of the workshop support the conclusion of the Life-Detection Science Conference, in particular the hypothesis-driven approaches and the need to start sample characterisation already during the sampling acquisition on Mars. The following conclusions are based on the sub-group and plenary discussions that occurred during the workshop. Most of them were identified in all of the individual sub-groups.

5.1. Conclusions on the general approach

1. A Planetary Protection Test Protocol should be data driven, i.e., responsive to the results of the individual or combined measurements. As a consequence, the sequence of experimental investigations and the application of pre-selected experimental techniques must allow some flexibility.
2. The same types of scientific measurements would inform the science and planetary protection elements.

3. A clear decision making framework, with well identified decision points is necessary to ensure the Earth safety aspect of planetary protection.

5.2. Conclusions in relation to sample analysis

4. The basic sequence of sample analysis should start with the analysis of martian material recovered from the outside of the sample containers, followed by head gas analysis, continued with the analysis of the samples while still retained in their individual containers, and finally conclude with the analysis of the samples after removal from their containers.
5. Sample preparation methods for individual investigations, in particular the preparation of thin and thick sections and remote manipulation of samples under containment, need dedicated planning and development efforts. This should involve the geological and paleontological science communities.
6. Extraction steps for molecular analysis, in particular wet chemistry, but also heat or a combination of both, need to be tailored and tested with analogue materials of the expected composition (e.g., based on previous *in-situ* missions or *in-situ* analysis during sample acquisition on Mars) and knowledge of the physical and chemical environment of the samples (e.g., presence of salts or oxidants) to ensure that molecular or cellular information is not destroyed and to demonstrate that the techniques work.
7. Terrestrial and/or synthetic analogue materials need to be developed and used as controls before and during the execution of the Planetary Protection Test Protocol.

5.3. Conclusions affecting the flight hardware design

8. Engineering of the sample storage system should facilitate an effective extraction of samples from the containers after return.
9. 3-D mapping of the samples, e.g., using X-ray tomography, prior to removing the individual samples from the sample containers is of critical importance for planning subsequent sample handling, sub-sampling, and analysis. Applying X-ray tomography on the samples would require a sample container made of a material with low atomic weight. Interfaces with facilities used to generate X-rays images of samples would need to be extensively tested.
10. Spectroscopic surface investigation of the samples while still in their containers would be complementary to the 3D X-ray tomography. This kind of observation would require a transparent window in the sample container.
11. Head space gas investigation before opening the sample containers requires a method for the extraction of gases that avoids fractionation and minimal mixing with the local external atmosphere.

The conclusions outlined above are the result of the discussions during the Life-Detection Workshop. They are intended to provide information for developing a science and planetary protection management approach for samples returned from Mars and to identify critical developments and flight system capabilities to enable the analysis of such samples.

It is recognised that the proposed sequence for sample analysis (Table 1) and the examples for the type of analytical methods used (Table 2) need to be further elaborated in the coming years.

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