



# **EURO-CARES**

## **Planetary Protection for Mars SR**

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# WP2 Objectives

- Ascertain and review planetary protection policies at a national and international level;
- To define methods, protocols and techniques for planetary protection issues and recommend protocols and methods for implementation.

# WP2 Science Team



|                 |      |                                            |    |                           |
|-----------------|------|--------------------------------------------|----|---------------------------|
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| Andrea Meneghin | INAF | Istituto Nazionale di Astrofisica          | IT | Engineer                  |
| Petra Rettberg  | DLR  | Deutsches Zentrum für Luft - und Raumfahrt | DE | Deputy                    |
| John Bridges    | LEI  | Leicester University                       | UK | Mars Sample Return        |
| Stefan Leuko    | DLR  | Deutsches Zentrum für Luft - und Raumfahrt | DE | Biologist                 |
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| John Holt       | LEI  | Leicester University                       | UK | Biohazard Technology      |
| Thomas Pottage  | PHE  | Public Ealth England                       | UK | Biosafety and Biosecurity |

# WP2 - Task 1.1

**Task 1.1:** Knowledge capture exercise of the main themes of the ESCF: Planetary Protection (INAF, PHE); Facilities and Infrastructure (NHMW, PHE); Instrumentation and Analytical Methods (MNHN); Analogue Samples (CNRS) and Portable Receiving Technologies (SEA)). A review of current and future sample return missions will also be carried out (NHML). This task will lead to a preliminary requirements document for each theme (D1.1-D1.6).

## **D 1.2 List of preliminary requirements for Planetary Protection**

Review of technology and science requirements in Planetary Protection and science contamination control;

Literature publications dealing with Planetary Protection issues for curation facility were summarized.



**D1.2 under revision due to 12<sup>th</sup> May 2016**

# PP Main Documents

## Mars Sample Return

- National Research Council, MSR: Issues and Recommendations (1997)
- National Research Council, An Astrobiology Strategy for the Exploration of Mars (2007).
- iMARS, Preliminary Planning for an International MSR Mission (2008).
- National Research Council, Assessment of PP Requirements for MSR Missions (2009).
- European Science Foundation *and* European Space Sciences Committee, MSR Backward Contamination - Strategic advice and requirements (2012).

# Planetary Protection

Two major contamination issues:

**Forward:** biological contamination of explored solar system bodies (false positive issues).

**Backward:** biological contamination of Earth as a result of returned extraterrestrial samples.

Science and mission requirements should cover any concerns about **sample return, landing site targeting and retrieval**, and **sample condition**.

# PP Requirements

Following ESA Planetary Protection Requirements (2012):

- Materials, including spacecraft surfaces exposed to the Martian environment, returned from Mars shall be treated as hazardous (i.e. severity category “catastrophic” as defined in ECSS-Q-ST-40C) until demonstrated otherwise.
- No uncontained Martian materials, including spacecraft surfaces exposed to the Martian environment, shall be returned from Mars unless sterilized.
- No portion of the materials returned from Mars shall be distributed unless a program of biohazard analysis, life-detection and sterilization is approved by the PP Officer.

# PP Requirements

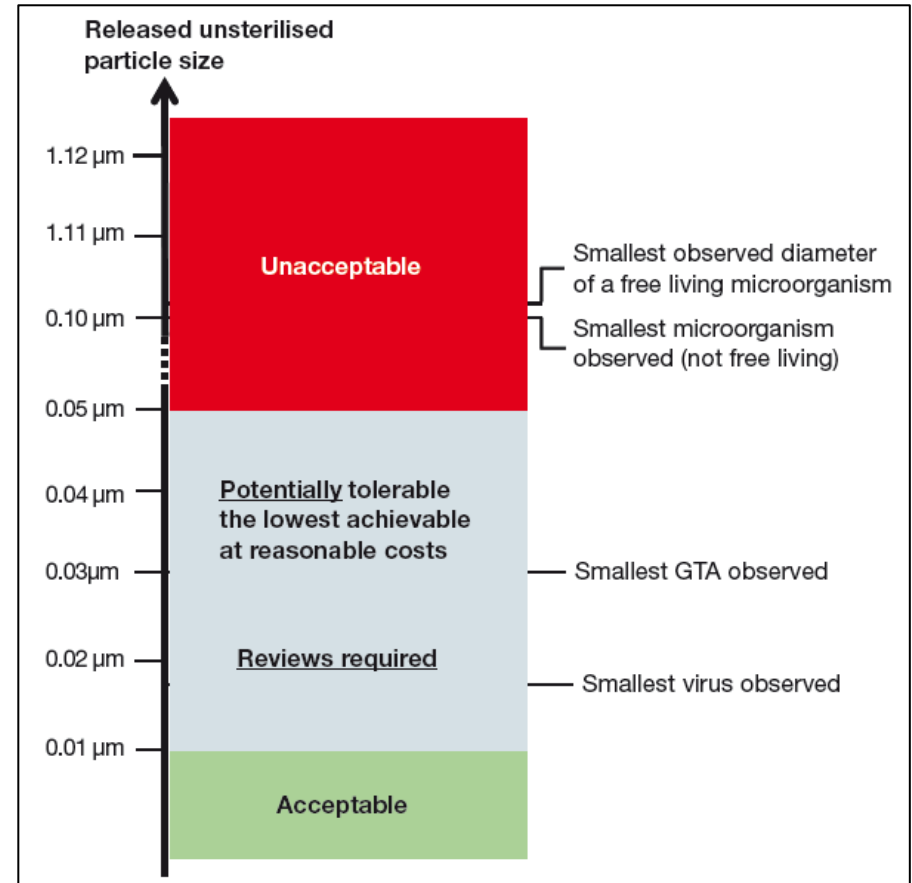
Regarding the probability of releasing of unsterilized particles, the ESF-ESSC document MSR Backward Contamination - Strategic advice and requirements (2012) make the following recommendations:

- The probability that a single unsterilized particle of  $0.01\ \mu\text{m}$  diameter or greater is released into the Earth's environment shall be less than  $10^{-6}$ .
- If the size requirement cannot be met without decreasing the overall level of assurance for the non-release of such a particle, the release of a single unsterilized particle of up to  $0.05\ \mu\text{m}$  can be considered as tolerable, assuming that it has been demonstrated that this size is the lowest achievable at a reasonable cost.
- The release of a single unsterilized particle larger than  $0.05\ \mu\text{m}$  is not acceptable under any circumstance.

# PP Requirements

**Table 2.** Smallest organisms

| Organisms                                                                        | Size                                                                    |
|----------------------------------------------------------------------------------|-------------------------------------------------------------------------|
| <b>Microorganisms</b>                                                            |                                                                         |
| Smallest bacteria:<br><i>Mycoplasma</i> species                                  | 0.1–0,2 $\mu\text{m}$                                                   |
| A theoretical coccoid cell with the minimum number of genes to be free living    | 0.15–0,2 $\mu\text{m}$                                                  |
| A theoretical rod-shaped cell with the minimum number of genes to be free living | Width: <0,1 $\mu\text{m}$<br>Length: >0,2 $\mu\text{m}$                 |
| Rods of “ <i>Pelagibacter ubiquus</i> ” archaea                                  | 0.12–0,2 $\mu\text{m}$ in diameter<br>0.37–0,84 $\mu\text{m}$ in length |
| Rods of <i>Thermophilum</i> bacteria                                             | 0.15–0,17 $\mu\text{m}$ in diameter<br>1–100 $\mu\text{m}$ in length    |
| <b>Viruses</b>                                                                   |                                                                         |
| Smallest virus observed:<br>single-stranded DNA porcine circovirus type 2        | 0,017 $\mu\text{m}$                                                     |
| <b>Gene Transfer Agents</b>                                                      |                                                                         |
| General GTAs                                                                     | 0.03–0,08 $\mu\text{m}$                                                 |



from *ESF-ESSC, MSR Backward Contamination - Strategic advice and requirements (2012)*

# Sample Containment

Returned samples can pose a risk for the Earth's biosphere, if they contain potentially hazardous agents.

Therefore **breaking the chain** of contact between the Mars environment and the Earth environment is necessary.

These concerns can significantly affect both flight and ground design operations (sampling mechanism, sample container design, ground recovery operations, SRF design, sample handling and control, biohazard-testing protocols, etc.)

# Sample Containment

The sample collection and containment gear would be **sterilized and isolated from other parts of the spacecraft.**

The isolation includes bio-barrier enclosures and modeling of contaminant migration patterns.

Another key development is a technique for collecting clean samples from beneath a Martian surface possibly contaminated by migration of microbes from a “dirty” lander or rover.

# Sample Containment

It has also to be considered that while some efforts to control the chemical cleanliness are also effective in reducing the bioburden, **others may work against the science goals.**

Ex: ethanol or isopropanol for cleaning surfaces are used to inactivate the majority of bacteria but they cannot be used for sterilization because they are not effective against bacterial spores and some other microorganisms. In addition, inactivated bacteria have lost their ability to replicate, but still represent a chemical contamination, if not removed from a surface.

# Spacecraft Sterilization

Unless the number of available sterilization techniques (physical, chemical, mechanical), for planetary protection ESA and NASA currently have only one accepted method of spacecraft sterilization: the **dry heat microbial reduction** (DHMR) process.

As an alternative, another technology was evaluated and accepted, the sterilization of appropriate elements of a spacecraft with **vapor phase hydrogen peroxide** (VHP).

# Sample storage facility

Pristine sample materials will not be removed from containment **until they are sterilized or certified as nonhazardous**, using a rigorous battery of life detection and biohazard tests.

Of the many PP technology that will be explored to design the curation facility, the cleaning and contamination control are **most directly linked to science requirements**.

# Sample storage facility

A [NASA protocol](#) for Mars returned sample handling exists in a draft form NPR 8020.12D (2011).

It has been a consistent theme during the development of the protocol that some effort must be made to analyze the physical/chemical characteristics of the sample as a prerequisite to life detection and biohazard testing and subsequent release of samples to the science community.

This involves PP issues.

# Sample storage facility

According to Kmineck (2014) the following steps for sample analysis has to be accomplished:

1. The sequence of sample analysis should start with the analysis of the 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.
2. 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.

# Sample storage facility

3. 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.
4. Terrestrial and/or synthetic **analogue materials** need to be developed and used as controls before and during the execution of the PP test protocol.

# Decisions about Release of Sample Material from Containment (NASA CP-2002-211841)

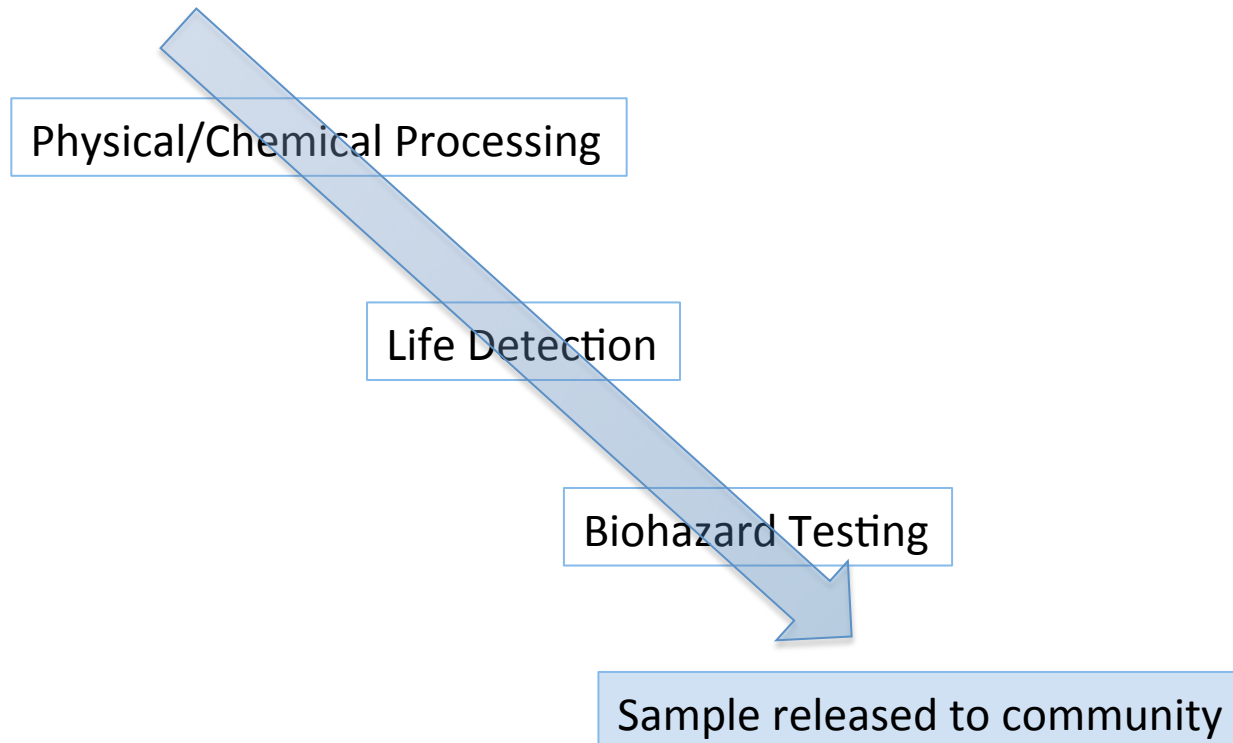


| Item | Question                                                                                 | Strategy                                                                                                                                                                                          |
|------|------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1    | Is there anything that looks like a life-form?                                           | Microscopy; Beam synchrotron or other non-destructive high-resolution analytic probe, particularly one that would allow testing unsterilized (yet still contained) samples outside main facility. |
| 2    | Is there a chemical signature of life?                                                   | Mass spec. or other analytical measurement systems (to be used in containment) that would identify biomolecules, chiral asymmetry, special bonding, etc.                                          |
| 3    | Is there any evidence of self replication or replication in terrestrial living organism? | Attempts to grow in culture, in cell culture, or in defined living organisms.                                                                                                                     |
| 4    | Is there any adverse effect on workers or the surrounding environment?                   | Microcosm tests; medical surveillance; evaluation of living systems in proximity of the receiving facility.                                                                                       |

# Sample delivery protocols

Before to deliver samples we have to ensure that a representative set of sub-samples undergoes sufficient testing to evaluate them against the release criteria.

Samples must be characterized, categorized, and analyzed to ensure that they can be sorted according to a procedure providing 'statistical relevance' to any sub-sampling.





This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement no 640190



# EURO-CARES

European Curation of Astromaterials Returned from  
Exploration of Space

**WP2 Meeting & Workshop**

## **Planetary Protection Aspects of European Sample Return Curation Facility**

June 14<sup>th</sup>–16<sup>th</sup>, 2016

University of Florence, Museum of Natural History – La Specola  
Via Romana 17, Firenze - Italy

The **WP2 Workshop and Meeting** will focus on the main Planetary Protection aspects related to European Sample Curation Facility, specifically:

- Techniques and methods of risk assessment of biohazard;
- Test methods for the detection of pathogenic organism;
- Containment systems;
- Sterilization processes;
- Safety equipment, enclosed containers, engineering control to remove or minimize to hazardous biological materials;
- Procedures to monitor the health of personal and select precautions for safe practice.

**For more information visit:**

**[www.euro-cares.eu](http://www.euro-cares.eu)**